PB82-232455

Interpretation of Low Resolution Mass Spectra for Level 1 Analysis of Environmental Mixtures

Arthur D. Little, Inc.
Cambridge, MA

Prepared for

Industrial Environmental Research Lab.
Research Triangle Park, NC

1981

U.S. Department of Commerce
National Technical Information Service
NTIS

PB82-232455

EPA 600/7-82-033

INTERPRETATION OF LOW RESOLUTION MASS SPECTRA
FOR
LEVEL 1 ANALYSIS OF ENVIRONMENTAL MIXTURES

Prepared For

EPA Project Officer: L. D. Johnson
Process Measurements Branch

Industrial Environmental Research Laboratory

Environmental Protection Agency

Research Triangle Park

EPA Contract No. 68-02-311

James L. Stauffer

Arthur D. Little, Inc.

Cambridge, Massachusetts 02140

C-82480-14

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U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

IERL-RTP-1328

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RECIPIENT'S ACCESSION NO. PB82-232455
4. TITLE AND SUBTITLE Interpretation of Low Resolution Mass Spectra for Level 1 Analysis of Environmental Mixtures		5. REPORT DATE
		6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) James L. Stauffer		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Arthur D. Little, Inc. Acorn Park Cambridge, Massachusetts 02140		10. PROGRAM ELEMENT NO.
		11. CONTRACT/GRANT NO. 68-02-3111, Task 113
12. SPONSORING AGENCY NAME AND ADDRESS **EPA, Office of Research and Development** **Industrial Environmental Research Laboratory** **Research Triangle Park, NC 27711**		13. TYPE OF REPORT AND PERIOD COVERED Task Final; 10/79-9/80
		14. SPONSORING AGENCY CODE **EPA/600/13**

15. SUPPLEMENTARY NOTES IERL-RTP project officer is Larry D. Johnson, Mail Drop 62, 919/541-7943. Supplements report EPA-600/7-78-201.

16. ABSTRACT

The report gives guidelines for interpreting the low resolution mass spectra (LRMS) of complex chemical mixtures, within the context of the EPA Level 1 Environmental Assessment Program. It discusses the principles underlying direct mass spectrometric analysis of complex mixtures, the techniques for optimizing the analysis run, and the interpretation and evaluation of the results. A chapter gives some interpretive aids for LRMS analysis of environmental mixtures. The guidelines are illustrated by a step-by-step detailed analysis of the mass spectra of four representative examples. The final chapter gives direction for reporting the results in the EPA Level 1 LRMS format. These guidelines supplement the overall description of Level 1 analysis methods given in the IERL-RTP Procedures Manual: Level 1 Environmental Assessment (second edition), EPA-600/7-78-201 (NTIS PB 293795).

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Pollution	Pollution Control	13B
Analyzing	Stationary Sources	14B
Mass Spectroscopy	Level 1 Analysis	
Chemical Composition	Low-Resolution Mass Spectrometry	07D

18. DISTRIBUTION STATEMENT **Release to Public**	19. SECURITY CLASS *(This Report)* **Unclassified**	21. NO. OF PAGES 161
	20. SECURITY CLASS *(This page)* **Unclassified**	22. PRICE

EPA Form 2220-1 (9-73)

ACKNOWLEDGEMENTS

This document has been submitted by Arthur D. Little, Inc., in partial fulfillment of the requirements of EPA Contract No. 68-02-3111, Technical Directive No. 113. Special appreciation is expressed to the Project Officer, Dr. Larry Johnson, and to Mr. J.A. Dorsey and Dr. R.G. Merrill, Jr., for their assistance in this task.

Appreciation is also expressed to the following individuals, who were kind enough to review and comment on the preliminary version of these guidelines: Mr. R.G. Beimer, TRW Systems; Dr. C. Lochmüller, Duke University; Dr. C. Miller and M. Kirk, Southern Research Institute; Drs. E. Pellizzari and W.D. Reynolds, Research Triangle Institute; and Dr. A.G. Sharkey, USERDA, Pittsburgh.

Special thanks are also extended to Drs. J. Harris, P. Levins, C. Rechsteiner and D. Sorlin of Arthur D. Little, Inc., for their review of the document.

ABSTRACT

This manual presents a set of guidelines for interpretation of the low resolution mass spectra (LRMS) of complex chemical mixtures, within the context of the EPA Level 1 Environmental Assessment Program. A discussion is given concerning the principles underlying direct mass spectrometric analysis of complex mixtures, the techniques for optimization of the analysis run, and the interpretation and evaluation of the results. A chapter is devoted to presentation of some interpretive aids for LRMS analysis of environmental mixtures. The guidelines are illustrated via a step-by-step, detailed analysis of the mass spectra of four representative examples. The final chapter gives direction for reporting the results in the EPA Level 1 LRMS format.

These guidelines are a supplement to the overall description of Level 1 analysis methods provided in the IERL-RTP Procedures Manual: Level 1 Environmental Assessment

TABLE OF CONTENTS

Page

I INTRODUCTION AND BACKGROUND 1

II ANALYSIS OF MIXTURES 11

A. Introduction 11
B. Experimental Techniques 18

1. LC Separation 18
2. Probe Microdistillation 22
3. Soft Ionization Techniques 31
4. Batch Inlet Analysis 36

C. Interpretation of Spectra 38

1. Interpretation of the Multicomponent Mass Spectrum 38
2. Reference Spectrum Match 42
3. Establishing Peak Associations 45

D. Confirmation Phase 48

1. Interpretation Checks 48
2. LC Fraction Interpretation Checks 48
3. IR Interpretation Checks 50
4. Spectrum Accounting 50

III INTERPRETATION AIDS 53

A. Introduction 53
B. The Correlation Tables 55

1. The Z Table 56
2. Probable Elemental Composition Assignments 58
3. The Chemical Class Tables 61
4. Chemical Class Parent-Fragment Relationships 64
5. Detection of Related Species or Classes 67
6. Tracing of Alkylated Species or Classes to the Base Ion 68

C. Polycyclic Organic Materials 69

1. The PAH Charts 70
2. The Aza Arenes 78
3. The Oxygen and Sulfur Polycyclics 82

TABLE OF CONTENTS (Continued)

Page

IV ANALYSIS EXAMPLES 87

A. Introduction 87
B. A Polar Mixture 88
C. A Simple Mixture 103
D. A Complex Spectrum 112
E. A Confusing Mixture 117

V THE LEVEL 1 LRMS REPORT 135

A. Introduction to the Report 135
B. Intensity Reporting 138
C. Molecular Weight Range Reporting 140
D. Category Reporting 141
E. Additional Categories 144
F. Subcategory and Specific Compounds Reporting 145
G. The Other Section of the Report 147
H. Examples 148

REFERENCES 153

LIST OF FIGURES

Figure No. Page

1 Low Resolution Mass Spectrometry Data Flow for Level 1 Analysis 10

2 Hypothetical Mass Spectrum Mixture Matrix Representation 14

3 Thermal Fractionation by Direct Probe Distillation 24

4 Extracted Ion Plots Showing Vaporization Maxima of Several Major Ions 26

5 Part I: Spectra Corresponding to Maxima of Figure 4 27

6 Part II: Spectra Corresponding to Maxima of Figure 4 28

7 Spectra Simplification Through Low Energy Ionization 33

8 Condensed Spectral Patterns 65

9 PAH Structural Series 75

10 Representative Oxygenated Polycyclics 84

11 Representative Sulfur and Oxysulfur Heterocyclics 85

12 Example #1: Mass Spectrum of a Polar LC Fraction 89

13 Spectrum Remaining After Subtraction of Tentatively Identified Components 99

14 Spectrum Accounting 104

15 Example #2: Spectrum of a Simple Mixture, LC Fraction 3 105

16 Example #3: A Complex Spectrum, Probe Temperature 80°C 113

17 Example #4: Cigarette Tar, LC Fraction 3, Probe Temperature 50°C 118

18 Example #4: Cigarette Tar, LC Fraction 3, Probe Temperature 60°C 119

19 Example #4: Cigarette Tar, LC Fraction 3, Probe Temperature 70°C 120

20 Example #4: Cigaratte Tar, LC Fraction 3, Probe Temperature 80°C 121

LIST OF FIGURES (Continued)

Figure No.		Page
21	Cigarette Tar, LC Fraction 2, Probe Temperature 60°C	122
22	Cigarette Tar, LC Fraction 2, Probe Temperature 70°C	123
23	Cigarette Tar, LC Fraction 4, Probe Temperature 60°C	124
24	Cigarette Tar, LC Fraction 4, Probe Temperature 70°C	125
25	Level 1 LRMS Report Form	136
26	Sample Level 1 LRMS Report, Example 1	149
27	Sample Level 1 LRMS Report, Example 2	150
28	Sample Level 1 LRMS Report, Example 3	151
29	Sample Level 1 LRMS Report, Example 4	152

LIST OF TABLES

Table No.		Page
1	Representative Chemical Class Distribution by LC Fraction	19
2	Information Elements	39
3	Matrix of M/Z Values According to Z Value and Minimum Carbon Number	57
4	Chemical Class By Z Value, LC Fraction 1 to 4	62
5	Chemical Class By Z Value, LC Fraction 4 to 7	63
6	PAH Nucleus List	72
7	PAH Composition, Structurally Ordered	74
8	Aza-PAH Nucleus List (N_1,N_2)	80
9	Peak Combinations for Library Search	93
10	Peak Combination for Library Search	94
11	M/Z 220 Library Search	96
12	M/Z 208 Library Search	97
13	Spectrum Accounting Matrix	102
14	Categories for Reporting LRMS Data	137

CHAPTER 1

INTRODUCTION AND BACKGROUND

The EPA Level 1 Environmental Assessment Program is the first phase of a three-phase source assessment program designed to address, "...to the maximum extent possible, the identification of all potential air, water and terrestrial pollution problems...".[1] The complete program has been planned for implementation in three phases in order to make most effective use of available resources, and to thereby also provide for the broadest coverage. The first phase, Level 1, is designed to produce a comprehensive survey of emissions from any industry or energy generating facility that might be of environmental consequence.[1] The results of this first sampling and analysis phase are utilized initially to judge whether or not a potential environmental hazard exists from a given source. The results also make possible a priority ranking for those sources which need follow-on analysis, and finally, provide the specific information that is utilized in the design and planning of the follow-on, more specifically directed Level II phase.

The sampling and analysis procedures of Level 1 are designed to yield quantitative final analytical results that are accurate to within a factor of 2 to 3.[1] For organic chemical components of the sample, qualitative results are to be reported by chemical class and molecular weight range, supplemented with any additional and more specific information that may be obtainable. Since it is not normally possible to predict for any source the complete nature of the materials to be analyzed, it is especially important that the analysis obtained by as comprehensive as possible. This, in turn, requires that the most universally applicable analysis methods be used.

In one or more of its many application modes, mass spectrometry (MS) is the most powerful and universally applicable technique available today for organic environmental analysis.[2,3,4] This is particularly true for trace analysis, for analysis of complex mixtures, or for analysis from a complex matrix. Since any or all of these conditions may apply to the samples to be analyzed by the Level 1 procedures, mass spectrometry has been chosen as one of the primary organic analysis techniques for the program. In order to best realize the universal detection potential of MS, the mode of operation used for the Level 1 procedure is low resolution mass spectrometry (LRMS), with sample introduction via both direct probe and batch inlet systems. The direct probe sample introduction method is suitable for a wider range of materials than gas chromatographic (GC) introduction can be, because it yields analysis before the sample undergoes hot surface collisions, and it is more sensitive to low vapor pressure materials. An illustration of the significance of the vapor pressure sensitivity is observable in the analysis of polycyclic aromatic hydrocarbons (PAH). It is well known that gas chromatography/mass spectrometry (GC/MS) analysis of combustion source samples rarely show PAH materials with molecular weights above 300; yet direct probe analysis of the same samples have shown that PAH materials with molecular weights ranging as high as 400 or 500 may in fact be as abundant as the lower molecular weight materials.[5]

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In order to obtain spectra of the more volatile (higher vapor pressure) materials, the batch inlet, with direct injection of the sample containing solvent, is utilized. The vapor pressure range of chemicals encountered in most source studies is such that both the direct probe and batch inlets are needed to complete analysis of a sample.

The instrumentation requirements for Level 1 LRMS are quite flexible, and have these minimum requirements:

1. Resolution of 1,000 (10% valley).
2. A batch inlet device with provision for direct injection of sample.
3. Direct insertion probe with controllable temperature.
4. Variable ionization modes: 70 eV electron impact, and either 15 eV electron impact or chemical ionization.
5. Electron miltiplier detection.

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Either direct oscillograph recording of the spectra or computerized data collection methods may be used. Each has advantages: the oscillograph recording yields more accurate isotope ratios and can give an indication of mass defects that is complimentary to the isotope ratios for elemental composition assignment; computerized data collection makes correlation of covaporizing peaks easier and usually permits a greater number of scans from a given sample.

For Page Numbering

LRMS for the Level 1 scheme differs in practice in several major respects from conventional MS analysis. The differences are brought about because:

(a) The sample is almost totally unknown prior to analysis;

(b) The sample is usually a mixture of components; and

(c) Primary reporting is by compound class.

This Guide addresses the special nature of LRMS analysis for the Level 1 scheme. The differences between Level 1 LRMS analysis procedures and those of conventional MS analysis have been developed primarily in response to the requirements of mixed special spectral interpretation. Many of the techniques discussed here are well proven in practice and may, taken individually, be considered to be conventional. Others have been developed especially for this Guide.

It is assumed throughout the Guide that the reader is familiar with single component MS analysis and that he or she is capable of obtaining an accurate identification from a sample of a pure compound via the technique of MS, from running the sample on the instrument through interpretation of the spectrum. Several excellent books have been published on these fundamental techniques. Of these, McLafferty's Interpretation of Mass Spectra[6] is perhaps the best known, and is probably also the most useful to the Level 1 LRMS analyst. Many of the techniques described therein are as applicable to mixture analysis as they are to the pure compounds for which they were developed. The reference tables supplied in the Appendix of that work can be particularly useful in Level 1.

The mass spectrometer has long been used as a tool for the direct analysis of mixtures. In the late 1940's successful routine analysis of mixtures with up to 30 components was reported (R.A. Brown[7] and references therein). Brown was the first to use mixed species mass spectra for the analysis of compound types rather than for individual compounds. His procedure, which involves the summing of the intensities of characteristic fragment ions for different hydrocarbon classes, became widely adopted in the petroleum industry.[8,9,10,11] Methods were developed using this approach combined with low voltage ionization for simplification of the spectral patterns.[12,13] Column chromatography for fractionation of the complex samples prior to analysis was also used.[14,19] These analysis methods were applied to mixtures that contained components largely of known type and composition range. The samples were introduced into the MS via a heated batch inlet that was generally kept at a constant temperature.

A major breakthrough in the capability for analysis of unknown composition mixtures occurred with the development of the direct insertion probe.[15] With the subsequent addition to the probe of independent and controllable heating, it became possible to differentially distill the components of a complex mixture directly into the ionization region of the MS source. This technique provides an initial separation stage for the components of the mixture, and is capable of yielding a set of spectra in which the relative concentrations of the individual components change systematically. This is the essential element for complete mixture analysis.[17,18] Grigsby[19] has reported a test case in which a 19 component aromatic mixture was subjected to comparative analysis via the direct probe and via capillary GC/MS. Both methods detected and gave virtually identical identifications for all 19 components. Each method detected additional, different compounds as impurities.

The other essential development that was necessary for successful routine analysis of unknown compounds was the compilation of large, indexed collections of reference spectra. Two primary sources of these are the *Eight Peak Index of Mass Spectra*,[20] and the *EPA/NIH Mass Spectral Data Base*.[21] For mixed species spectra, the principal route to sample identification is through the use of reference spectra. It is essential in this context to have a well indexed reference spectrum library which can be searched on the basis of the two or three major ions of a spectrum. While many forms of indexing can be used, the most useful for mixture analysis is that ordered on ion intensity. Molecular weight ordering subordered on ion intensity is almost equally useful and occasionally better than the direct ion intensity ordering. In this regard, the *Eight Peak Index of Mass Spectra* is ideal in that it has both forms, as well as a molecular weight tabulation that is subordered on composition.

The EPA/NIH Mass Spectral Data Base is also very useful, although somewhat slower to use, as it is not indexed on the major ions of the spectra. It is, however, ordered on molecular weight, subordered on composition, so that the spectrum of a tentatively identified component can be quickly located for comparison with the unknown. Since all of the spectra are graphically displayed, total spectrum comparison is somewhat facilitated as compared to a digital presentation.

An important element for successful analysis of unknown composition mixtures by LRMS is supplied in the Level 1 analysis scheme by liquid chromatographic (LC) fractionation of the samples prior to MS analysis. The fractions so obtained are analyzed individually by LRMS, using both the batch inlet for direct solution injection, and the direct insertion probe for a dried down aliquot of the solution. The essential effect of the LC separation is to provide fractions for LRMS analysis that are each a subset of the total mixture, separated according to polarity.

A major additional contribution to the interpretation of the Level 1 LRMS spectra of multicomponent mixtures can be provided through knowledge of the source being sampled. While it is clearly necessary to keep an open mind as to what may appear from any source, the nature of the facility being sampled does suggest an open set of plausible materials that may be observed. An understanding of the "likely" materials will often provide the initial key to understanding a complex mixed species spectrum. Two studies that illustrate effective utilization of sample source information as a primary spectral interpretation aid are those of Hites and Lopez-Avila,[22] and Games and Hites.[23] These papers are exemplary in their demonstration of the way in which knowledge of the source can be incorporated into the interpretation of the spectra.

Some other analysis techniques, complimentary to the LRMS data, provide additional information that can be important to interpretation of the LRMS spectra. Infrared (IR) analysis of each LC fraction provides potentially important qualitative information. Quantitative information is obtained from gas chromatographic analyses (total chromatographable organics, TCO) for the volatile, and by gravimetric analyses (GRAV) for the non-volatile portions of the sample. Information from all of these sources is integrated in the organics analysis results report after the analysis is completed, but most of it is also directly important to the evaluation and interpretation of the LRMS spectra.

The supplemental information that is of principal importance to LRMS interpretation is the data obtainable from the IR. The IR data are complimentary to the LRMS data in two major respects. First, the IR is more oriented toward recognition of functional groups than is MS, and second, it is not vapor pressure limited. The IR data can alert the MS analyst to specific classes of compounds prior to interpretation of the LRMS data, and it can and should be used to provide confirmation or rejection of the identifications made via LRMS. Although the MS has inherently greater sensitivity than the IR, the IR has the potential to detect some materials that may be overlooked by MS. This is due to the comparatively greater response that the IR has to a few functional groups, and to its ability to analyze materials in the condensed phase.

The TCO and GRAV values obtained on each LC fraction are indicative of the concentration and comparative vapor pressures for that fraction, guiding the analyst as to suitable sample size for analysis, and providing an absolute basis for estimating quantities. The GC profile obtained from the TCO analysis can also be very useful in correlating species identifications.

The LRMS report for Level 1 is the product of many interactions, both within the data obtained by LRMS and with that obtained by the other techniques. The reliable identification of specific compound classes (and sometimes specific compounds) from within a complex matrix is achieved by combining many clues of lesser specificity. When all agree, the identification can be considered quite reliable. If some sources of information confirm the identification, but others are ambiguous, the assignment can range from probable to possible. If some sources contradict, the classification must be changed or reclassified as unknown.

Figure 1 summarizes the interactive nature of the analysis and provides an overview of the context in which the analysis is performed.

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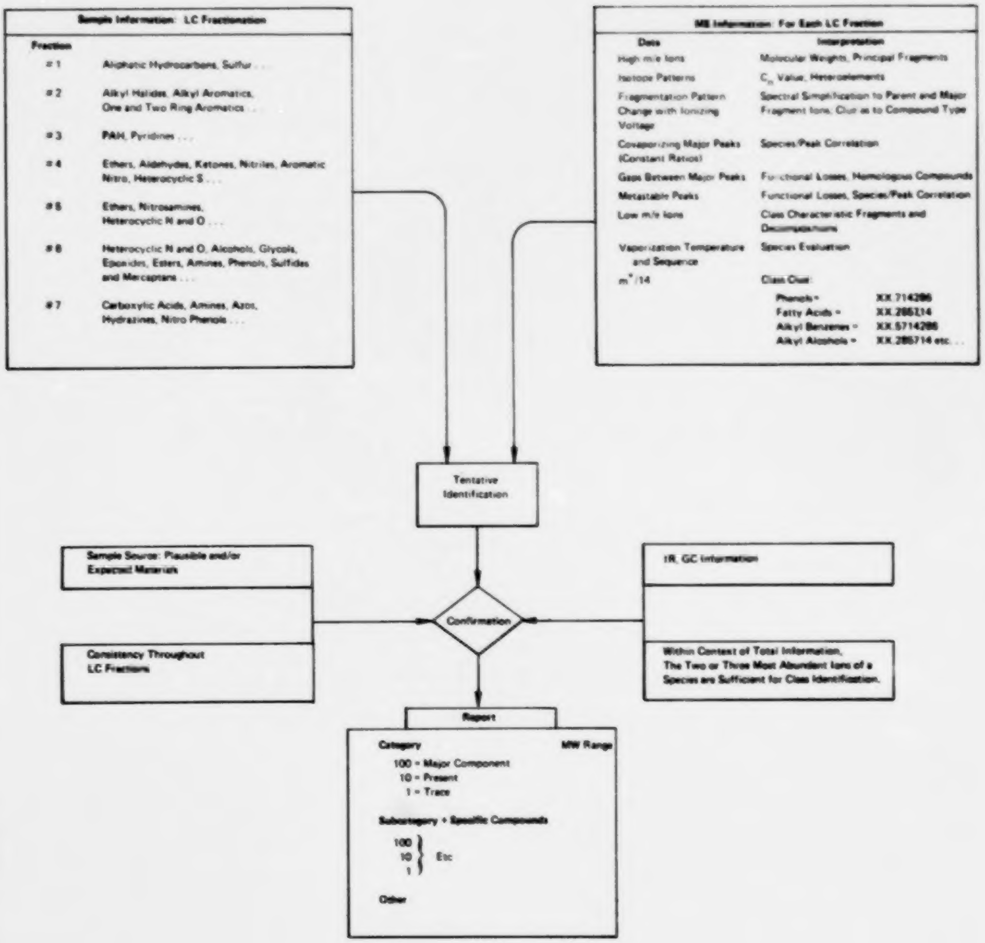
Sample Information: LC Fractionation
Fraction
# 1 Aliphatic Hydrocarbons, Sulfur . . .
# 2 Alkyl Halides, Alkyl Aromatics, One and Two Ring Aromatics . . .
# 3 PAH, Pyridines . . .
# 4 Ethers, Aldehydes, Ketones, Nitriles, Aromatic Nitro, Heterocyclic S . . .
# 5 Ethers, Nitrosamines, Heterocyclic N and O . . .
# 6 Heterocyclic N and O, Alcohols, Glycols, Epoxides, Esters, Amines, Phenols, Sulfides and Mercaptans . . .
# 7 Carboxylic Acids, Amines, Azos, Hydrazines, Nitro Phenols . . .
MS Information: For Each LC Fraction
Data
Interpretation
High m/e Ions
Molecular Weights, Principal Fragments
Isotope Patterns
C_n Value, Heteroelements
Fragmentation Pattern
Change with Ionizing Voltage
Spectral Simplification to Parent and Major Fragment Ions, Clue as to Compound Type
Covaporizing Major Peaks (Constant Ratios)
Species/Peak Correlation
Gaps Between Major Peaks
Functional Losses, Homologous Compounds
Metastable Peaks
Functional Losses, Species/Peak Correlation
Low m/e Ions
Class Characteristic Fragments and Decompositions
Vaporization Temperature and Sequence
Species Evaluation
m*/14
Class Clue:
Phenols = XX.714286
Fatty Acids = XX.285714
Alkyl Benzenes = XX.5714286
Alkyl Alcohols = XX.285714 etc. . .
Tentative Identification
Sample Source: Plausible and/or Expected Materials
Consistency Throughout LC Fractions
Confirmation
IR, GC Information
Within Context of Total Information, The Two or Three Most Abundant Ions of a Species are Sufficient for Class Identification.
Report
Category
MW Range
100 = Major Component
10 = Present
1 = Trace
Subcategory + Specific Compounds
100
10
1
Etc
Other

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FIGURE 1 LOW RESOLUTION MASS SPECTROMETRY DATA FLOW FOR LEVEL 1 ANALYSIS

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CHAPTER II

ANALYSIS OF MIXTURES

A. Introduction

The detail that may be resolved from the mass spectrum of a multicomponent mixture is dependent both on the complexity of the spectrum itself, and on the amount of supplemental information that is available. The precision of the identifications that may be obtained will vary accordingly, ranging from specific compound or composition assignments for all of the spectrum, to simply an indication of the chemical classes that are present. In the first case, the components of the mixture may be identified with an assurance that is only slightly lower than that obtainable from separate spectra of the individual components. At the other end of the range, the spectrum can be so complex that the LRMS analyst is required to make extensive use of supplemental information in order to be able to specify the major chemical classes of the mixture. Nevertheless, the accuracy of the compound class assignments for the latter case may be comparable to those for the first, even though the specificity of the assignments is much broader. Since the requirements for LRMS in the Level 1 program are for identification to the level of chemical class and for quantification to a factor of 2 or 3, both cases described may satisfy those requirements.

The accurate identification of chemical class is not a trivial task, but it is an achievable goal for the great majority of samples that are analyzed within the Level 1 program, since the LRMS data are supported by other information. This supplementary information comes principally from the LC fractionation scheme, the IR analysis, and the known characteristics of the source. Nevertheless the LRMS data remain the principal source of qualitative organic chemical information, as they are both the most specific and the most comprehensive available. In order to perform the evaluation that is necessary to obtain this information, it is necessary first to understand some of the fundamental characteristics of the mass spectra of multicomponent mixtures.

The mass spectrum of a multicomponent mixture is the arithmetic sum of the mass spectra of the individual components of that mixture. The individual spectra are in effect superimposed in a manner that is, to the first order, additive only. Thus, no destructive interferences or shifts of mass values occur as a result of the combination of the separate elements. Hence, in one important sense, it is correct to say that no information is lost. The information has, through the superimposition of the several spectra, been rendered less accessible than it would be in the separate spectra, but it is still present as a part of the total spectrum of the multicomponent mixture.

This rather remarkable fact is true because mass spectra are principally made up of elements that are fundamentally discrete in nature. The major information elements of a spectrum are the m/z or mass values of the individual peaks, the ratios of the intensities of the peaks that are present, and the absence of peaks at other values. The interrelationship of these three principal mass spectral elements is entirely dependent on the chemical nature of the ionized material that gives rise to the spectrum, and independent of the intrinsic value of any of the individual spectral elements. Consequently, when the mass spectra of different chemical species are combined, the discrete values of the individual spectra are simply added, over a common mass scale. The mass spectrum that will be obtained from a 10 component mixture is essentially identical to that which would be obtained if the individual spectra of those same 10 components were obtained separately, in proportion to their concentrations in the first mixture, and then arithmetically added together.

This behavior may be illustrated through the example of a mathematical matrix as shown in Figure 2. In this hypothetical representation, each row of the matrix would be the mass spectrum of a single component of the mixture. There are as many rows as there are components in the mixture, and as many columns as mass values in the unknown spectra. The spectrum that would be obtained directly from the multicomponent mixture would be equal to the columnar sum of the matrix. The information contained in the spectra of the individual components has been combined into the single composite spectrum of the mixture. The information has in one sense not been lost, inasmuch as it has all been transferred to the composite spectrum. But the composite spectrum does not contain any information regarding the number of rows that have contributed to its summed value, or to the proportions of those contributions, except m/z values

Species	a	b	c	d	e	f	g	h	i	j
A	45			30			100	10		
B			5		10		20		100	10
C	50				100			30		
D			20			60			100	5
Columnar Sum (observed mixture spectrum)	95	--	25	30	110	60	120	40	200	15

FIGURE 2 HYPOTHETICAL MASS SPECTRUM MIXTURE MATRIX REPRESENTATION

for those mass values that have zero intensity. The information from the individual components is present in the composite spectrum only as a check sum; it can be used to confirm the fit of a complete set of spectral assignments, but not directly to discover what those assignments might be. If the proper combination of individual spectra can be discovered, the confidence that may be placed in the assignments of those spectra need only be slightly less than if they were separate, individual spectra. The certainty with which spectral identification assignments can be made from the spectra of multicomponent mixtures will of necessity always be somewhat lower than that obtainable from the spectrum of a single, pure compound. But the difference between the two is one of degree, not of kind. There is no essential difference in the rationale underlying the association of spectral features and chemical characteristics, whether the spectral features be from the spectra of one or of several components. The limitation inherent in the analysis of multicomponent mass spectra stems from the difficulty of finding the set of correct spectra that will, when summed, yield the observed total spectrum.

The task confronting the analyst of the mass spectra of multicomponent mixtures is to discover the correct combination of individual spectra which, when placed into the rows of the matrix, will yield a columnar sum that is equivalent to the observed multicomponent spectrum. If each individual datum throughout the matrix had to be found individually, it would, in most cases, be impossible to determine the original configuration that would give rise to the observed multicomponent spectrum. The feature that makes it possible to discover the original combination is the fact that a tentative identification assignment determines values for a whole row of the matrix. The tentative identification may be a specific compound, or it may be a compound class. In either case, the rest of the spectrum (or row) must be consistent with that tentative identification, and the row must fit completely into the checksum that is the observed spectrum. This requirement of overall fit and consistency, when fulfilled, assures a high degree of reliability for the identifications. It is important to realize that this is true whether the identifications are for specific composition and structural types, or whether they are for chemical classes.

The identification determined in the LRMS analysis should also be consistent with the supplementary information for the sample. There are many potential sources for such information in a complete LRMS analysis. Not all will be available in any given analysis, but in most cases there will be enough for identifications that are commensurate with the needs of the Level 1 program.

The most important source of supplemental information for the initial interpretation of a multicomponent mass spectrum is additional spectra of the same set of components, obtained under differing conditions. The most useful of these are the spectra obtained from the probe due to differential distillation of the various components of of the mixture. This results in changing the proportion of the individual components in successive spectra. The fact that several components are vaporizing simultaneously does not weaken the usefulness of this information, provided that the proportions of these several components change from scan to scan. This is the normal case during probe sample analysis. Any change of sample or analysis conditions between different scans taken from the same complex mixture can, through the difference between the two spectra, yield information about the sample that is not available in either of the single scans. It is, in fact, intrinsically possible to mathematically completely resolve the superimposed spectra of a mixture into the separate spectra of the mixture components, if the set of spectra of the mixture contains at least one more scan, than the number of components in the mixture.[17,18] However, this degree of analysis is not ordinarily practical within the context of the Level 1 program, nor is it generally necessary. It is usually possible, given such a set of spectra, to directly perceive spectral features that can provide the necessary keys for resolution of the mixture.

The next most important stage of supplemental information can be obtained through the use of soft ionization techniques, such as chemical ionization (CI), or low voltage electron impact ionization. The set of spectra obtained during distillation of the sample from the probe can provide an effective basis for recognition of the major individual components of the mixture, but if the mixture is complex, minor components may remain hidden unless soft ionization techniques are used for some of the scans. The spectral simplification that is obtainable through the

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use of soft ionization can be very effective in revealing minor components of complex mixtures. The abundant fragment ions of major components may easily mask the much smaller parent ions of minor components. When these large and often numerous fragments are eliminated through the use of soft ionization, the minor component parent ions become much more observable.

B. Experimental Techniques

1. LC Separation

Before the sample is analyzed by LRMS, it is subjected to an LC separation scheme,[1] which divides the sample into 7 fractions. The fractions are then individually analyzed by LRMS and IR. The LC separation is of fundamental importance in the interpretation of the mass spectra, as it limits the possible range of materials that may appear in any one fraction.

A list of the distribution of some chemical categories by LC fraction is shown in Table 1. This list is representative rather than definitive, and is based on past experience. Experience has also shown that both the distribution and the separation of the components are dependent on LC column conditions, technique, and sample concentration.[38]

The LC separation is principally determined by species polarity; the materials eluting into the fractions range from non-polar compounds in

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TABLE 1

REPRESENTATIVE CHEMICAL CLASS DISTRIBUTION BY LC FRACTION

LC FRACTION		
1. aliphatic hydrocarbon, sulfur	halogenated aliphatics halogenated aromatics	
2. aromatic hydrocarbons	aromatic hydrocarbons	heterocyclic sulfur, PAH
3. PAH species		
4. carbazoles	nitroaromatics	heterocyclic N heterocyclic O ketones esters
5.		
6.	carboxylic acids phenols sulfonic acids	
7.		

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fraction 1 to most polar in fraction 7. The resolution is most complete for the early fractions, becoming progressively less so in the later ones. The early fractions will occasionally show some overlap of organic compound types, the later ones almost always. This is particularly apparent for samples of high concentration. Although this overlap does complicate the spectrum of any given LC fraction where it occurs, it also may be used to the analyst's advantage.

There are three aspects of the LC separation scheme that are of major importance to the LRMS analyst. The first aspect is simply that the LC fraction is a subset of the total sample, and therefore less complex and correspondingly easier to interpret than would be the total sample. The second aspect is the inclusion and exclusion feature of the fractions, which frequently allows one to chose between materials that have similar mass spectral characteristics. The third aspect is the information that can be obtained through observation of fraction interrelationships.

There are many examples of dissimilar materials which give rise to mass spectra with similar features. If these dissimilar materials belong to different chemical classes, they will elute in different LC fractions. Consideration of this separation is of fundamental importance in the interpretation of Level 1 LRMS spectra. The behavior of aliphatic hydrocarbon chains can be used as an example.

Aliphatic hydrocarbons give rise to a characteristic mass spectral fragmentation pattern. This pattern will appear whenever aliphatic hydrocarbon chains are present, whether they be part of a mixture as independent species, as a major substituents on aromatic nuclei, or when they occur in combination with other functional groups. In the mass spectra of single chemical components, there are enough distinguishing features for these materials to be accurately identified. But when the mass spectrum is that of a mixture, it is quite possible that the distinguishing features will be obscured, or simply difficult to identify. The LC fraction can give the analyst an important clue in such cases--if a characteristic aliphatic spectrum appears in fraction number 1, it is reasonable to assume that it is due to aliphatic species. But if an "aliphatic" spectrum" appears first in fraction number 3, without having appeared in fraction number 2, then the analyst should look for evidence of aliphatically substituted aromatic material in fraction number 3. If the "aliphatic" spectrum appears somewhere in the later fractions, the analyst should look for evidence of polar substituents (alcohols, fatty acids, amines, etc.), or for aliphatically substituted polar polycyclic material.

The spectra of aliphatic hydrocarbon constitutents may also be used as an example to illustrate one aspect of the use of LC fraction interrelationships. If from a given sample it is observed that LC fractions 1 and 3 both contain significant aliphatic fragment ions, but fraction 2 does not, then it is reasonable to assume that the aliphatic components of fraction 3 must be aliphatic substituents of some other species, and to look for evidence of that. If on the other hand, the aliphatic character had been present in all three fractions, with fractions 2 and 3 each containing less than the previous fraction, one might reasonably suspect an overlap in the LC fractionation. This possibility could be confirmed or rejected through the use of soft ionization, as the parent ions of aliphatic species would, if present, most likely be observable.

Another use of LC fraction interrelationships can arise when a species is not all contained in a single fraction, but overlaps into two or more. In these instances the spectrum of the overlapping compound will appear in a different spectral matrix in each fraction. Therefore, features that may have been difficult to detect in the mass spectrum of one fraction may be readily observed in another.

These examples are only representative of some of the ways in which consideration of the LC fraction characteristics may be utilized in the interpretation of the mass spectra. Since there are many opportunities of this sort, it is frequently beneficial to defer interpretation of the mass spectra of a Level 1 sample until spectra for all of the fractions of that sample are available.

2. Probe Microdistillation

Probe microdistillation during LRMS analysis is the most important separation technique under the control of the spectroscopist.[24,25,26] The change in comparative concentration of the several components of the vaporizing mixture with each scan provides the essential information for recognition and measurement of those components. For accurate evaluation of all of the components present in a mixture it is in principle necessary to have a number of scans (of differing relative concentrations) that is at least one greater than the number of components.[17,18] The degree of mixture evaluation necessary for Level 1 chemical class and molecular weight range identification may not always require this many unique scans. However, a large number of spectra helps assure that the behavior of the sample vaporization is followed closely. The fewer the spectra taken, the greater the possibility that the vaporization maximum of a given species will occur between scans. When this occurs, it can give rise to many interpretation problems: the intensity of the species may be misrepresented; the species may not be recognized; other species may be misidentified. Too few spectra also may render the correct association of molecular and fragment ions very difficult or impossible, since one of the principal ways this information is determined is by the observation that ions are co-vaporizing.

A graphical example of the sequential vaporization of an aromatic fraction is shown in Figure 3. The total reconstructed ion current (RIC) plot illustrating the overall vaporization of the sample is shown in the lower part of the figure. The upper "three dimensional" part of the figure shows ion intensity profiles for each m/z value between 100 and 450. Temperature increases with time and scan number along the X axis;

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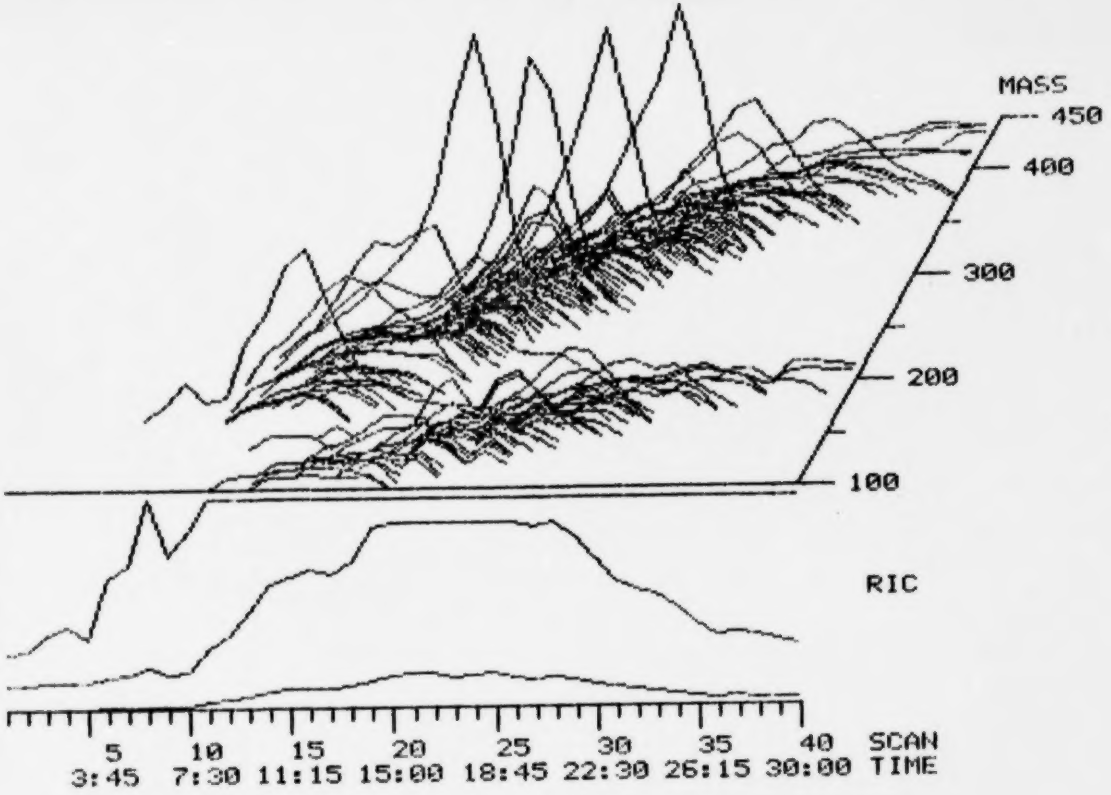

FIGURE 3 THERMAL FRACTIONATION BY DIRECT PROBE DISTILLATION

mass values increase toward the "rear" of the plot. This three dimensional plot illustrates the thermal fractionation of the sample. The lowest molecular weight materials vaporize first, at the lower temperatures. Then with increasing time and temperature, successively higher molecular weight materials appear, maximize, and are exhausted. Ion intensity profiles that have similar shapes and which in addition have simultaneous maxima, have a high probability of belonging to the same species spectrum. Profiles which overlap in time but which maximize at different times and have different shapes, may belong in part to the same individual species spectrum, but at least one of the ions must have contributions from an additional species. Profiles that do not overlap in time must arise from differing chemical species. In the example shown in Figure 3, most major ions are clearly from different species. Near the middle of the display two pairs of ion profiles of lesser intenstiy appear simultaneously which are similar enough to belong to the same species. The lower intensity peaks in the 100 to 200 mass range are from double ionization of the parent ion molecules.

Another example of a vaporization sequence is shown in Figures 4, 5, and 6. Figure 4 is a computer generated series of extracted ion chromatograms of m/z values 149, 167, 217, 252, 276, and 304, obtained from the analysis of a coal tar LC fraction 4 sample. The data were taken from a 50 scan run using the direct insertion probe. The sample was introduced into the source at scan 9, with the source temperature at 80°C. The temperature was raised at an approximately even rate to the maximum temperature of 320°C, reached near scan 50. All spectra are

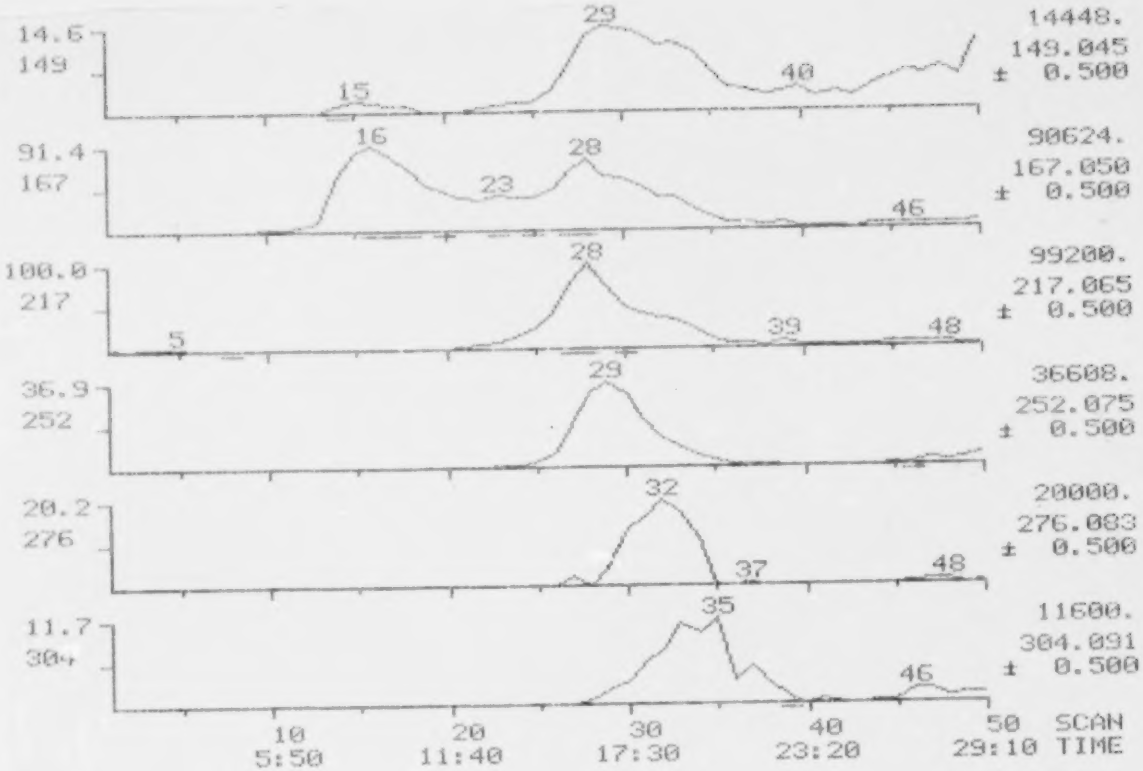

FIGURE 4 EXTRACTED ION PLOTS SHOWING VAPORIZATION MAXIMA OF SEVERAL MAJOR IONS

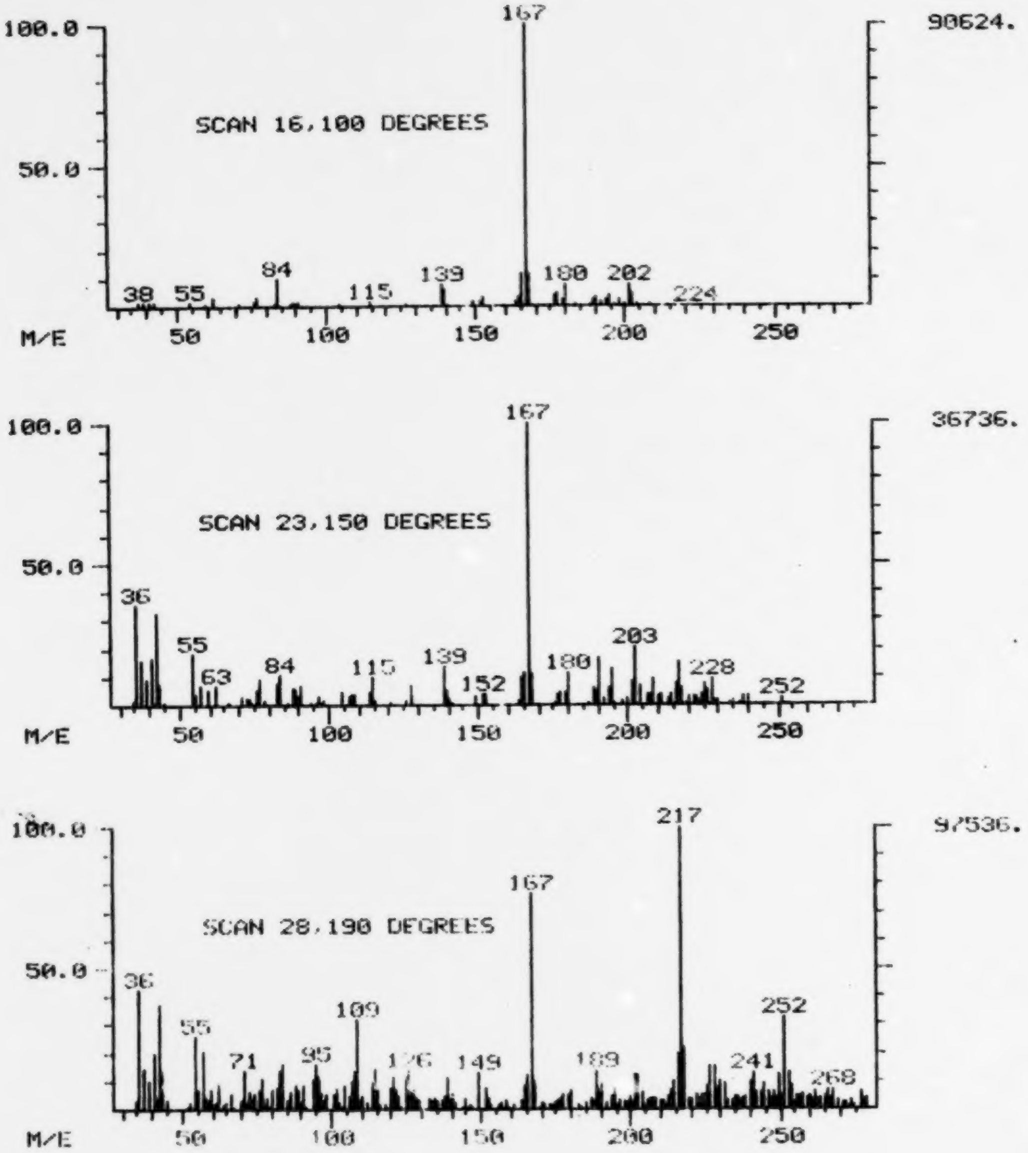

FIGURE 5 PART I: SPECTRA CORRESPONDING TO MAXIMA OF FIGURE 4

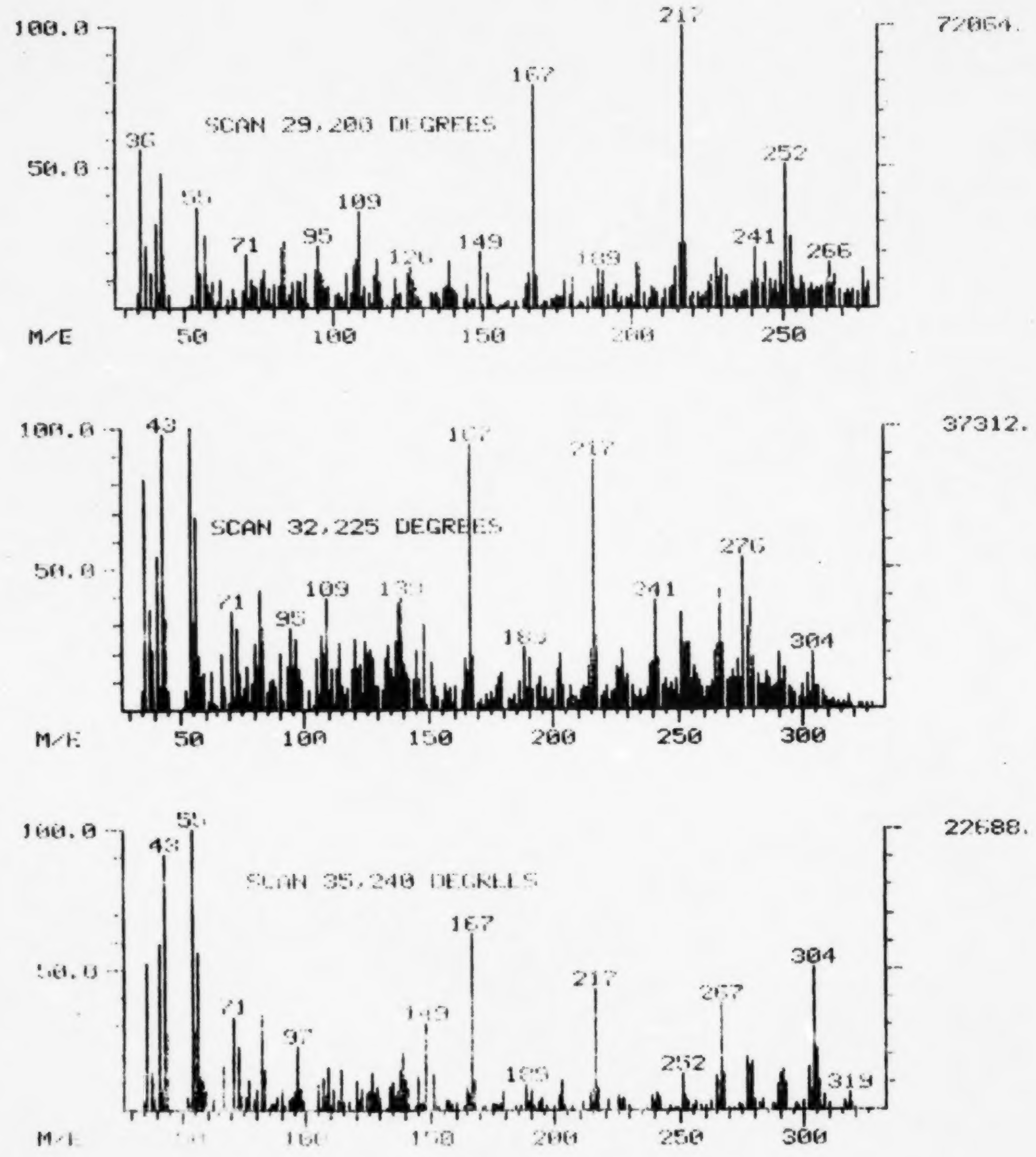

FIGURE 6 PART II: SPECTRA CORRESPONDING TO MAXIMA OF FIGURE 4

electron impact, taken at 70 eV. Individual vaporization curves of Figure 4 have been normalized to full scale on each plot; the absolute size of each is indicated on the right side of the plots, and the sizes relative to m/z 217 as 100% on the left side. The extracted ion plots indicate the vaporization sequence of each ion, with the scan numbers of maximum intensity labeled on each plot. Spectra corresponding to each of these maxima are shown in Figures 5 and 6.

It is advisable to use more spectra for characterization of a sample than the six shown in Figures 5 and 6, but these six spectra are enough to illustrate the technique. The changes in the mass spectra from 100° to 240°C indicate that this sample contains several species, both major and minor in concentration. The separation is less than complete, with overlap between species being apparent. The essential thing to obtain from thermal fractionation, however, is not complete separation but rather enough separation to: (1) be able to detect that more than one species is present; and (2) be able to find at least two correlating ions with which to begin the identification procedure.

It is apparent, first of all, that m/z 167 must represent something different than most of the rest of the peaks, since it is present almost by itself at the lower temperatures. The next major peak to appear is m/z 217, along with m/z 252 and m/z 109. Over the range of spectra in which all three appear, m/z 252 changes relative to m/z 217 (100%), from 33% up to 50% and then down to less than 30% at scan 35. On the other hand, m/z 109, is about 33% relative to m/z 217 in all scans except 32, where it is slightly higher. This indicates that m/z 217 and m/z 252 probably represent different species, with m/z 252 showing a maximum vaporization rate at a slightly higher temperature than that for m/z 217. However, m/z 109 appears with one exception to share the same vaporization curve as m/z 217, and so may very well be from the same species. Further consideration suggests that m/z 109 may be the doubly ionized peak from m/z 217, rounded off 1/2 m/z high by the computer.

By analogy, one might then also associate the m/z 126 peaks with the m/z 252, and the m/z 138, 139 pair with m/z 276 and 278. A low ionizing voltage scan taken during this period of apparent double ionization would, if this interpretation were correct, completely eliminate the doubly ionized peaks. As a minimum, however, the series of thermally fractionated 70 eV spectra have allowed association of a second peak with each of four possible molecular ions. These secondary, associated peaks can be used for initial reference spectrum matching.

Discovering peak associations that correlate major peaks as being likely to have come from the same species, is one of the major keys to the analysis of mixtures. Two major ions are enough to key a library search that will yield few enough possible matches to make evaluation of those matches feasible; but three ions obviously yield much greater specificity, and are to be preferred if they can be obtained.

The temperature range used for the probe portion of the analysis should be as wide as possible. The minimum or starting temperature should generally be well below 100°C, and ideally would be close to the temperature used for solvent drydown. Exception may be made of it is known in advance that no volatile (TCO) material was detected, and if in addition the MS is operated in a rapid scanning mode so as to catch any early vaporizers. However, starting temperatures significantly above 100°C are likely to lead to spectra that are difficult to interpret, due to excessive instantaneous vaporization of sample. Spectra well spread out in time are generally the most interpretable.

It is advisable to heat the sample to as high a temperature as the probe system can safely sustain. There is no information available before LRMS analysis to indicate the appropriate upper temperature limit for the sample, and any fraction may contain high molecular weight (less volatile) material. Lack of sample vaporizing in the middle part of the temperature range, for example, does not reliably indicate that there will be none in the high temperature range.

3. Soft Ionization Techniques

Soft ionization modes of source operation (CI, low eV EI) are, for resolution of the components of the mixture, second only to probe microdistillation in importance. The spectral simplification that is obtainable through the elminination of fragment ions by the use of soft ionization will almost always be found to be of considerable use in the interpretation of mixed species spectra. Since soft ionization reduces or eliminates entirely the peaks due to fragmentation, it produces spectra from a given mixture that are much simpler than those taken with 70 eV electron impact ionization. In some respects, these simpler spectra may be much easier to interpret than their complex counterparts. Minor components are detected more easily. Comparison between soft ionization and 70 eV electron impact spectra can readily establish many parent-fragment relationships that can be important to spectral interpretation. This section discusses low voltage EI only, but the example of spectral simplification would also apply to CI. The molecular ions would, of course, have different mass values, usually either M+1 or M-1.

Soft ionization modes are usable throughout the temperature range of the probe, though high source temperatures may degrade CI performance somewhat. The disadvantages involved in their use are that the absolute sensitivity at low ionizing voltages is usually lower than at 70 eV, and that the observed envelope of vaporization for any given species is altered when the ionization mode is switched. This alteration renders interpretation of thermal fractionation somewhat more complex, but potentially gives more information overall.

A direct example of spectral simplification using 15 eV EI is shown in Figure 7. The two spectra are taken from the same probe analysis run of a coal tar LC fraction 5 sample. The two plots are each normalized to 100% for the base peak of the spectrum (left side), with the absolute intensities shown on the right side of each spectrum. It can be seen that in this case the 15 eV ionization mode has reduced the absolute sensitivity

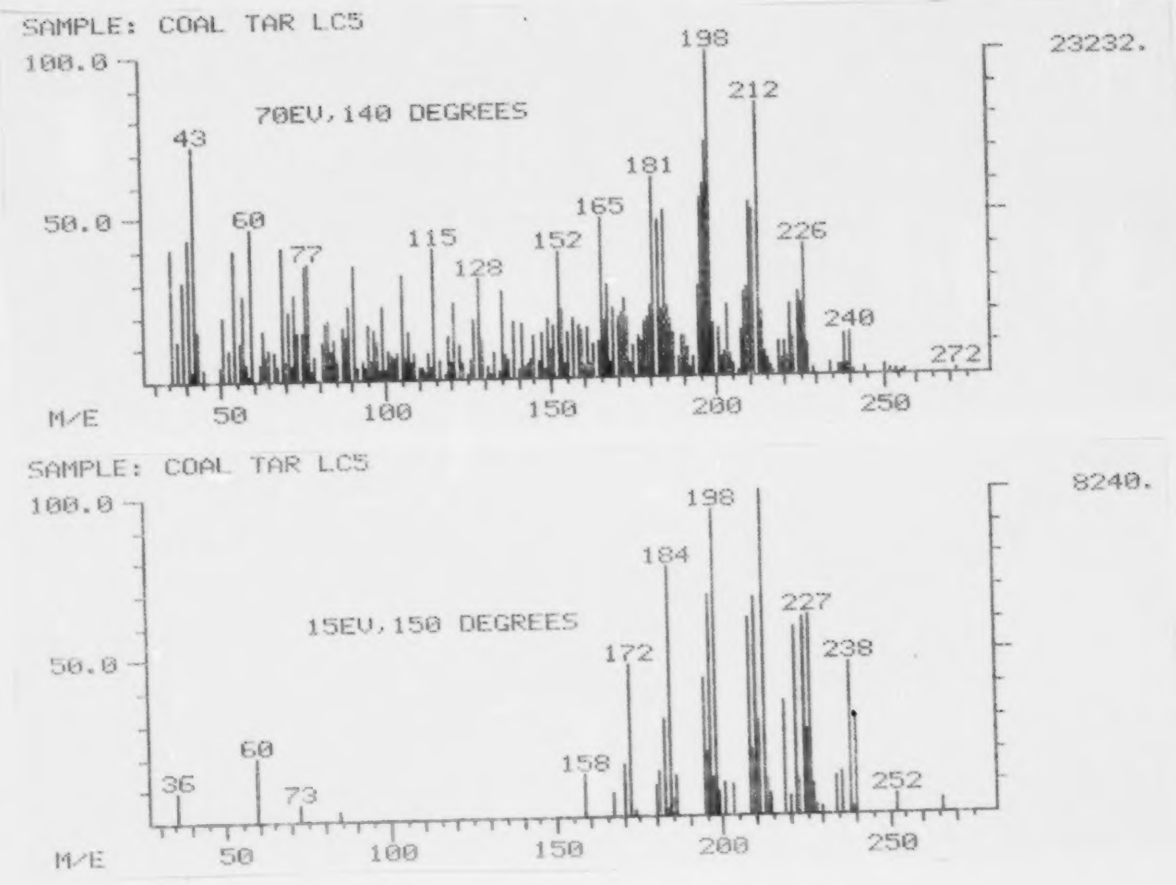

FIGURE 7 SPECTRAL SIMPLIFICATION THROUGH LOW ENERGY IONIZATION

by about a factor of 3 (8,240 vs. 23,232), but at the same time has produced a dramatic improvement in the readability of the spectrum. It is immediately clear that most of the ions of the 70 eV spectra are identifiable as fragments. This is a particularly important piece of information for the 70 eV spectrum ion peaks at m/z 181 and 195. Otherwise they might be interpreted as alkyl carbazoles, whereas in fact they are fragments of higher molecular weight materials. But, it is also important to know that m/z 115, 128, 152, and 165 are fragment ions. The 15 eV spectrum identifies the molecular ions, while the 70 eV scan, by comparison, illustrates the fragment ions which are needed for reference spectrum matching. Structurally indicative fragements are abundant in the 70 eV spectrum: m/z 77, 91, 105 point toward the presence of substituted benzenes; m/z 121, 135, and a small 107 suggest that an alkylated phenol may be present. On the other hand, m/z 60 and 72 persisting at 15 eV suggest that a COO function of some type may also be present.

It is clear that the combination of the two spectra greatly facilitates interpretation compared with either one alone. The ideal situation might be to have the ionization mode switched on alternate scans between 70 eV and the low voltage value, so that there might be a complete set of both kinds of spectra. This ideal state is probably unworkable on most existing equipment, so one must instead be content with doing most of the analysis in one of the two modes, switching to the other as seems appropriate. In a temperature region that shows high sample vaporization rates and much complexity, it may be suitable to alternate the ionizing voltage setting between low and high voltage every 30 seconds or so; the most suitable rate for switching will be determined by the scan rate used on the MS, the sample rate of vaporization and its complexity, and the refocus requirements of the MS source.

The most useful value for the low voltage setting needs to be established for each instrument. Mass spectrometers intended for general analytical work do not usually have sources that give accurate energy values at low ionizing potentials. This is not a significant handicap, for what is needed is not an accurate measurement of an appearance potential, but merely a substantial reduction of the fragmentation produced by 70 eV electrons. (The effective ionizing voltage at low energies may be roughly calibrated by observing the apparent appearance potential of the common background ions, such as N_2^+, O_2^+, Ar^+, CO_2^+, etc.) The system may be tuned by observing the spectrum of known materials as the ionizing voltage is reduced. The ionizing voltage value that gives the most abundant parent ion while at the same time minimizing the fragment ions is most appropriate. It will probably fall somewhere between 10 eV and 20 eV. Different classes of compound will show different optimum values; aliphatics need the lowest voltage and for that reason as well as for their very extensive fragmentation, are not good candidates for the experiment. Highly aromatic compounds such as the PAH materials are also not very well suited to the experiment, as they show almost no fragmentation, even at 70 eV. They do show show substantial peaks from double ionization, as do most other compounds of high structural stability; the double ionization shows a much higher appearance potential, usually over

20 eV. The double ionization peaks can serve as valuable correlation peaks for the PAH materials, which otherwise give such a simple spectrum that species support information is sometimes hard to find in their spectra.

Inasmuch as Level 1 samples represent something approaching a true unknown and ordinarily are run only once, a middle value for the low eV setting is probably most appropriate. It is reasonable to tailor values to some extent during the run. If LC fraction 1 is observed to be predominantly aliphatic, a very low eV setting (10 to 12 eV) needs to be used for any significant simplifying effect to take place. LC fraction 3, on the other hand, would show unnecessarily low sensitivity at those values; if it is observed to be predominantly PAH type material, a value in the range of 15 eV to 20 eV would be suitable.

In all cases, spectra run at low voltage should be repeated at 70 eV with as little time lapse as possible, as the 70 eV spectra are needed for reference library comparison. In addition, the 70 eV spectra have a much higher information content than do the simpler soft ionization spectra. This greater information content is of considerable importance for accurate identification assignments from multicomponent spectra.

4. Batch Inlet Analysis

LC fractions are obtained as solutions; before they can be run on the direct insertion probe, the solvent must be removed. This is usually accomplished by evaporation, which unavoidably leads to loss of the more volatile components. Even room temperature drydown of solvents may result in substantial loss of materials with boiling points lower than 300°C. In addition to the losses during sample drydown, loses can occur in the vacuum lock of the direct insertion probe and, if the source temperature is too high, also on sample insertion.

In batch inlet analysis, the sample solution is injected directly into the batch inlet, which effectively eliminates the loss of volatile components. However, sensitivity from the batch inlet is comparatively poor--the sample remains diluted in the solvent, and the flow into the MS is restricted. The sample size limits are set by the pressure tolerance of the source. The amount of material that can be injected depends on the inlet volume, leak flow rate, and source pumping speed, and so will be very instrument dependent. Generally the maximum injection size will be on the order of a few microliters, with concentration requirements on the order of a microgram of sample per microliter of solvent. Thus, the detection limit from the batch inlet is a per component concentration of about 0.1%. The probe on the other hand, is not concentration limited, as the amount of sample (solvent) that can be dried down is essentially unlimited.

Even with the comparatively poor sensitivity of 0.1%, lower levels of volatiles can be detected via the batch inlet than from the probe. Little time need be spent on the acquisition of data from the batch inlet, as it is a steady state device and all that in principle is needed is a single spectrum, though practice will usually dictate a few scans be taken in order to insure that it is indeed at a steady state.

When an LC fraction yields complex spectra from the batch inlet, the spectra may be difficult to interpret since there is no separation process analogous to probe microdistillation. In these cases, the GC plot from the TCO analysis can provide valuable information regarding the number of components present, and the proportions between those components.

C. Interpretation of Spectra

1. Interpretation of the Multicomponent Mass Spectrum

Interpretation of mixtures via LRMS is a highly iterative process in which clues of many kinds, both from the LRMS information and from other Level 1 analytical data, are assigned, intercompared, and reassigned, if necessary, until data from all sources support the final interpretation. Though it may be that no one information element possesses sufficient specificity for a positive identification, the combined evidence of many separate sources of data will act to greatly enhance the probability of a correct assignment. The range of assignements that will agree with all of the information elements is normally narrow enough to unambigously establish the compound categories, even from a mixture. Frequently it will also be possible to identify sub-categories, or even specific compositions and compound types, although the exact isomers may remain unknown.

A list of some potential information elements that can be useful in these analyses is shown in Table 2. Most of these, including those listed under MS, are independent elements of information. It is fitting at this point, to recall that the mass spectrum of a single species is an assemblage of independent elements that are linked together only by the

TABLE 2

INFORMATION ELEMENTS

MS	m/Z value
	parent ions
	appearance potential
	metastable peaks
	major ions (fragments)
	neutral losses (spectrum gaps)
	fragmentation pattern
	fragmentation pattern variation with eV
	peak intensity
	peak intensity ratios
	isotope ratios
	double ionization
	co-vaporization of peaks
	differential vaporization of peaks
	vaporization temperature
	vaporization sequence
	R&DB value
	homologous series
	Z value
LC	LC fraction number
	adjacent LC fraction spectra
IR	functional group assignment
	specific compound confirmation
TCO	absolute (gram) values
	GC curve
GRAV	absolute (gram) values
SAMPLING	sampling train characteristics
SOURCE	expected chemical characteristics of source sampled

characteristics of the species being analyzed. LRMS identification in general is based on many independent elements of information, each indicating only a comparatively small group of possiblities. An identification is made when there is a possiblity that is common to all elements. This process usually defines a unique or nearly unique material.[27]

The major difficulties of mixed spectrum analysis are of two kinds:

1. Masking of the spectral patterns;
2. Specific ion interferences.

Masking of the spectral pattern arises from the superimposition of several compound or spectrum types. It is potentially the most serious problem for this kind of analysis, as enough different spectrum types could overlay one another to render the combined spectrum essentially featureless. Fortunately, this degree of concealment is very rare--in the course of a probe run there are enough changes of relative intensities so that intercomparisons of spectra will allow one to perceive some pattern. Once a start has been made whereby something can be selected for tentative identification or initial sorting, the remaining part of the spectrum is simplified. The part that has been tentatively classified should be subtracted from all of the spectra in which it appears in that run; this will both simplify all of those spectra, and provide confirmation for the classification through repeated observations and correlations of the pattern.[28]

Specific ion interferences result from the superimposition of ions from different sample compounds, appearing at the same nominal m/z value. It can occur both from mixing of compound or spectral types, and from different species of the same compound or spectral type. In the latter case it will usually enhance the visibility of the ions characteristics of the class, through addition of those class characteristic ions. A very small contribution to a specific m/z peak from one species is likely to be lost, in effect, if there is a large simultaneous contribution to that ion from a different species. However, it is infrequent that such small peaks are important to an identification. The principal difficulty presented by this interference is to recognize that it has occured; the additive components may be readily misinterpreted as being abundant ions from a single species. Such masking can reliably be detected only by peak ratio comparisons after some identifications have been made, though it is sometimes possible to see such small peaks directly. For these reasons mixture analyses concentrates on the major ions, except when minor ones that appear to have high specificity are present. Usually this means ions of high molecular weight, especially when they appear to be molecular ions.

The principal modes of single species mass spectrum identification, namely spectral interpretation and reference spectrum match, are also fundamental to the analysis of mixed species spectra. Some modification of primary techniques needs to be made in adapting them for use in multi spectral and multicomponent analysis, but the basic approach remains very much the same in both.

2. Reference Spectrum Match

A reference spectrum match is usually accepted as the most authoritative identification possible by MS, especially if both spectra are run on the same instrument. Such a match involves a comparison of virtually all of the peaks in the spectrum, and so might seem to have little use for mxiture analysis; in fact, it is quite possibly the most useful single technique for the task. Tentative identifications are usually made on the basis of only two or three most intense high molecular weight ions--this establishes a range of possible compounds that are subsequently reverse matched to the mixed spectrum. Discrepancies in the reverse match due to missing or undersized peaks in the unknown spectrum will eliminate most of the set, so that only a brief list remains for further consideration.

The most useful formats for indexing of libraries of reference spectra that are to be used for LRMS Level 1 analysis are those ordered on the most abundant ions in the spectrum, and those ordered on molecular weight, subordered on the most abundant ions. Both are typically keyed to the three largest peaks; conveniently, this represents about the number of peaks that it is possible to correlate from a series of mixed spectra. Inasmuch as mixed species spectra are subject to distortions from specific ion interferences, allowance for substantial variation of intensity range must be included in the initial stages of reference matching. Such interferences are most common at low m/z values; the initial efforts at correlation are therefore directed primarily at the higher m/z values. The higher m/z ions also generally have appreciable higher species specificity than the low m/z ions.

Once two or three ions have been established, through procedures such as those described below as probably being associated, the index can be searched for possibilities. If the two or three ions are fairly close in intensity in the unknown, the library should be searched for all possible orders of intensity of the major ions. For two ions:

A, B
B, A

for three ions:

A, B, C
A, C, B
B, A, C
B, C, A
C, A, B
C, B, A

If there is a factor of 10 or so difference in intensity in the unknown ions, it is probably satisfactory, initially at least, to search the library using only the observed order of abundance. That is, if peak A is about 10 times as large as B, only those entries in which A is larger than B need be searched.

The reference spectra that are selected by this search are then compared on a peak by peak basis over the remainder of the spectrum. Any

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TOP

peaks present in the reference spectrum that are not observable in the unknown will disqualify that reference spectrum as a possible identification. Peaks in the unknown that are larger than their counterpart in the reference spectrum do not disqualify the identification, since there is a possibility of additive interference.

Intensity evaluation throughout the spectrum should be in proportion to the relative intensity ratios observed on the major, indexing ions. That is, if the intensity ratios of the two or three major ions of the reference spectrum agree fairly closely with the unknown, it is reasonable to assume that the rest of the spectrum should be proportionally accurate; this criterion establishes minimum acceptable values. If the major ion ratios are substantially different from the unknown, even though perhaps of the same order of abundance, there is an implication that there must be an interfering material present for the identification be be valid. Consequently, the criterion for acceptable intensity ratios for the rest of the spectrum must be adjusted in proportion to the error in the major ion intensity ratios.

Intensity level considerations of this detail can normally be left to the final stages, if they become necessary at all. It is usually sufficient to drop from the list of possible correlations those reference spectra that have significant peaks that are missing from the unknown. This process will by itself usually reduce the list of possibilities for each set of major peak associations to not more than a very few spectra, which can then readily be evaluated via other information elements.

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TOP

This process will, of course, not guarantee that the list of possibilities developed actually contains the correct identification for the unknown--but it should contain all of the possible identifications that are present in the reference library.

3. Establishing Peak Associations

In order to identify the peaks within the spectra that are to be grouped together for reference spectra comparison, peak associations need to be established. In this context peak associations are those peaks which appear to come from the same material, usually from a single species. Since reference spectra are of single species it is necessary to seek correlation on that level; the correlations found will usually be less than complete due to the complexity of the mixture, but will also usually be more than adequate for the chemical compound class level of identification.

10 Lines to End of Typing Area

Peaks may be correlated through observation of their vaporization temperatures, ionization behavior, and by consideration of possible fragmentations between them. It is usually most advantageous to attempt to establish associations for only one or two classes at a time; if this is successful, the identified spectrum can then be subtracted from the unknown mixture, thereby simplifying it for further evaluation.

If two or three peaks can be established as co-vaporizing, there is good reason to suspect that they may be from the same species. The fragmentation pattern (proportions) of a single species will not change

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significantly during the period of vaporization from the probe. Consequently, the peak height ratios of co-vaporizing peaks are a good indicator of possible associations. If the peak height ratios between the co-vaporizing peaks remain the same throughout the course of their vaporization, then it is very likely that they are of the same species and therefore prime candidates for a library search. However, if the peaks co-vaporize but show intensity ratios that differ measurably over the course of their vaporization, they either must be from different species, or one or more of the peaks must have more than one species contributing to it. Peaks that do not co-vaporize must be from different species.

Comparison of low ionizing voltage spectra with normal 70 eV spectra can also be very helpful in evaluating peak associations. Some fragmentation processes cease entirely at low ionizing voltage, leaving only the parent ion or at least much simplified spectrum of that compound. Most species will shown a noticeable reduction in the intensity of the fragment ions compared to the molecular ion. When this can be observed, it is possible to establish definite associations between molecular and fragment ions in the 70 eV Spectrum

The simplest use of low eV spectra results from those cases where nothing remains in the spectrum but molecular ions. The molecular ions thus identified can then be correlated with the major fragment ions in the 70 eV spectrum without excessive speculation. It must be remembered that fragments from two or more species that vaporize simultaneously will be reduced or eliminated together; the fact that all are fragments does not indicate that they are necessarily of the same species or class. The correlations of molecular and fragment ions must include not only co-vaporization behavior at 70 eV, but also feasible neutral fragmentation losses.

Spectral interpretation considerations for the establishment of peak associations are primarily speculations as to probable neutral losses, usually of relatively small functional units at comparatively high m/z values. Spectra that otherwise escape correlation of peaks through insufficient scans or lack of low voltage spectra may often be correlated by searching for plausible neutral losses. Simple arithmetic differences are taken between major peaks and between all significant peaks at high mass; the numbers so obtained are then correlated with possible neutral fragments. A list of representative neutral fragments is available in McLafferty's Interpretation of Mass Spectra,[6] along with a list of common fragment ions. (The identification potential of fragment ions is much more completely developed in Mass Spectral Correlation, also by McLafferty.[29])

The example of a polar mixture worked through in Chapter IV of this Guide, makes extensive use of the neutral fragment loss technique to establish peak associations.

USE THIS SHEET FOR REPORT TYPING 8-1/2 x 11

TOP

D. Confirmation Phase

1. Interpretation Checks

All of the procedures described to this point yield identifications that should be considered tentative until they can be supported by complementary techniques. Even the use of much more sophisticated instruments than LRMS does not eliminate the need for complementary techniques when complex samples are being evaluated.[30] Only in the unusual case of not more than one or two non-interferring species being present in the LC fraction, can the identification be established unequivocably without additional support. The more usual route is to first seek tentative identifications from the spectra obtained for the fraction, utilizing whatever supplemental information may be availble. One must then take those tentative identifications and test them against all of the other information obtained on the sample from both LRMS and the other techniques. In the first round, the other sources of information may be used to suggest possible identification, or to provide clues to that end. In the confirming phase specific though tentative identifications are tested against the other sources. In addition, all of the identifications need to be tested against each other for internal consistency.

2. LC Fraction Interpretation Checks

10 Lines to End of Typing Area

The phase of testing identifications against each other begins with LC fraction comparisons. Initial assignments are sometimes facilitated through comparison of the spectra from LC fractions adjacent to that being analyzed; in a similar manner, adjacent fractions can provide supporting information to the tentative identifications. Adjacent fractions may show the same or a similar compound at a concentration different from

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that presented in the fraction being analyzed, and in a different matrix. When this occurs, the first assignment is reinforced by the additional observations. There will usually be some coherence to the species observed across the series of LC fractions that will also support the interpretation. However, the lack of such a sequence should not necessarily be taken as a negative indication, as it may be the result of the sample and not the identification.

The spectra identified within the LC fraction must show features consistent with the proposed interpretation, such as vaporization temperatures in the correct boiling point sequence from one species to another, co-vaporization of all identifiable ions from a species (not just those used initially for reference spectra search), and the correct isotope ratios where they can be measured without interference.

Content considerations from the LRMS spectra of a single LC fraction can also reinforce identifications. Homologous compounds when present give increased credence to the identifications of each, particularly when present in proportions that are typical for the sample source. This is especially true for alkylations, some of which can be expected to show regular intensity variations. Such preconceptions must, however, be used with care--that is, with additional independent support--to minimize prejudging of the sample. However, relationship considerations of this sort are not inappropriate when comparing samples collected from different parts of the SASS train on a given sampling run, as these series of samples may be expected to show a fairly regular progress of components from one end of the train to the other.

3. IR Interpretation Checks

One of the most important sources for confirmation of tentative identifications is the IR data. It is important because it is not only an independent source, but also one with a significant degree of specificity. It can, and should, be used at two levels:

1. to confirm the presence of functional groups and compound classes
2. to confirm the presence of specific compounds,

There are, of course, several possibilities in addition to confirmation at these levels. The IR may give no information regarding a particular proposed identification because of weak bands and/or interferences from more abundant components. The IR spectrum may, by the absence of an expected absorption band, contradict a tentative LRMS identification. Conversely, the IR spectrum may contain some features that are not satisfactorily accounted for by the set of compounds/classes tentatively identified by LRMS. In the case of conflict between techniques, it is not generally appropriate to conclude that one information source is correct and the other wrong. The interpretation of either or both may be incorrect, and both should be reexamined in the case of apparently conflicting data.

4. Spectrum Accounting

An evaluation needs to be made of how completely the proposed identifications account for all of the ions in the observed spectra. This is done by reverse matching of all of the identified components of an LC

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fraction to the spectra of that fraction. In some cases, all peaks in the spectra will have been adequately accounted for. In other cases, the presence of previously undetected components may be indicated by peaks remaining unaccounted for after the reverse match. For complex mixtures, reverse matching can probably be done adequately for the higher mass region only, due to much greater variability of the low end of the spectrum.

This reverse match may also serve to identify any major specific ion interferences. Somewhat more attention needs to be paid to relative peak intensities at this stage than was done in the tentative identification phase. It is necessary to make some allowance for instrumental variations in this comparison; the ratios observed for the major ions of the species can serve as a guide in this regard. Two such peaks can be used, but three major peaks are clearly to be preferred, as the probability for three peaks showing a specific ion interference is much smaller than for two. Unless an instrument is known to exhibit a particular bias, an error of greater than about 30% in the ratios of the major peaks should be considered indicative of a possible interference or a mistaken identification. The most reliable indication of additive interferences occurs when one or two peaks out of a spectrum are out of proportion, while the rest of the spectrum is well matched. When these out-of-proportion peaks are too large, they may contain ions from a different species, or the spectrum may be misidentified. If the out-of-proportion ions are too small for the rest of the spectrum, the identification is probably wrong. Discrepancies of this sort need to be carefully checked out with the IR data.

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1279 200M

CHAPTER III

INTERPRETATION AIDS

A. Introduction

In order to meet its intended purpose, the Level 1 analysis scheme must approach as nearly as is practically possible a universal detection scheme. The system must be capable of dealing not only with materials that may be expected to be in the effluent, but also with those that are totally unexpected. Since mass spectrometry is inherently perhaps the most universally applicable single technique available for organics analysis, it has a very important role in the Level 1 program. The LRMS analyst can be seen to have a major responsibility for the detection and evaluation of unexpected materials. This requirement for universal detection tends to limit the potential applicability of analysis aids for LRMS, as such aids inherently involve some preliminary assumptions about the samples to be analyzed.

There are two possible approaches for dealing with this limitation: (1) the least specific and most universal aids can be used, and (2) more specific and consequently more powerful aids can be applied if in their use it is continually recognized that they are only a partial answer. Probably the most efficient and effective analysis can be attained when both approaches are used interactively.

The first category, that is, the least specific and most universal aid, is simply the largest reference spectrum library available. It is

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the most important aid to the LRMS analyst as most of the identifications attainable will be based on matches to reference spectra. But the most complete collection of reference spectra will quickly be found to be incomplete for the analysis of effluent samples, or to be inadequate in some respects for mixture analysis. Thus, there will frequently be a need for spectral interpretation over and above that obtainable from reference spectra. The techniques that were detailed in the previous chapter can be made more effective when they are supplemented by inputs from specific sample source information and generalized analysis aids. The generalized analysis aids are the subject of this Chapter.

The interpretive aids included in this chapter are intended to be an aid for recognition of types of material, not an independent authority. They are not a substitute for reference spectra, or the associative considerations discussed in Chapter II and illustrated in Chapter IV. When used as intended they may provide a useful aid to the analyst, and offer a significant time saving service, but they are not suitable for use in a stand alone fashion.

The tables presented in this section are very condensed in order to offer quick and wide correlations that may give clues as to possible identifications, which in turn, can be evaluated through reference spectra. Since effluent samples will usually be only partially covered by reference spectra, it will generally be necessary to make some interpolations from similar compounds, or from homologous species. Although the techniques described in Chapter II do have some usefulness in this

USE THIS SHEET FOR REPORT TYPING 8-1/2 X 11

TOP

regard, they are not particularly effective in finding spectra of related materials. The interpretation aids of this Chapter are intended in part for that purpose. They are designed to point quickly to possible classes of compounds and, correspondingly, also to homologs within a class.

The correlation feature of relating mass spectral peaks to possible chemical types can also be of use in relating the IR data to the LRMS information, in both directions. When the IR has identified a functionality that has not been detected by LRMS, the tables may be used to reference that function to specific mass peaks for further study. Conversely, candidate compound classes that are signaled by the tables from the LRMS data can be correlated to the IR information.

B. The Correlation Tables

The first group of analysis aids, made up of three tables and a chart, together form one set of interpretation aids. In order to simplify the description of the aids, they are introduced one at a time. However, they are designed as a set, and so will probably be most effective for most uses when used as such, on an interactive basis. A few of the potential uses for the set are:

(1) probable elemental composition assignments;
(2) quick selection of possible chemical classes;
(3) elucidation of parent-fragment relationships;
(4) detection of related species or classes; and
(5) tracing of alkylated species or classes to the base ion.

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For Page Numbering

1279-200M

Descriptions are given for each of these uses. In the process of following the descriptions or in applying the techniques, the analyst will probably discover additional uses for the set.

1. The Z Table

The Z value of a hydrocarbon composition is a measure or indication of the degree of unsaturation of that composition. The Z value is given by the relationship:

$$mw = C_nH_{(2n + Z)}$$

Thus an alkane has a Z value of +2; a monoalkene, a Z value of 0; an alkylbenzene, a Z value of -6, and so on. Only a very limited range of Z values are possible for any single m/z number. Consequently, a direct correlation to a limited range of possible chemical classes can be made through consideration of the possible Z values of an m/z number. The relationship is most definitive for molecular ions, but it is often useful for fragment ions as well.

Table 3 (the Z table)[31] is a compilation that gives the possible Z values, and minimum carbon number, for all m/z values between 45 and 366. Each column of the table may be correlated with a limited range of chemical classes. Hydrocarbon m/z values for any given column must have one of the Z values listed at the top of that column. Thus a hydrocarbon ion that has an m/z number that appears in the right most column of the table must have a composition with a Z value from the series +2, -12, -26, etc. The Z values shown at the top of the table are only the first

USE THIS SHEET FOR REPORT TYPING 8-1/2 x 11

TOP

TABLE 3

MATRIX OF M/Z VALUES ACCORDING TO Z VALUE AND MINIMUM CARBON NUMBER

Z Value

	-11 -25 -39	-10 -24 -38	-9 -23 -37	-8 -22 -36	-7 -21 -35	-6 -20 -34	-5 -19 -33	-4 -18 -32	-3 -17 -31	-2 -16 -30	-1 -15 -29	0 -14 -28	1 -13 -27	2 -12 -26
C #								AMU						
4	45	46	47	48	49	50	51	52	53	54	55	56	57	58
5	59	60	61	62	63	64	65	66	67	68	69	70	71	72
6	73	74	75	76	77	78	79	80	81	82	83	84	85	86
7	87	88	89	90	91	92	93	94	95	96	97	98	99	100
8	101	102	103	104	105	106	107	108	109	110	111	112	113	114
9	115	116	117	118	119	120	121	122	123	124	125	126	127	128
10	129	130	131	132	133	134	135	136	137	138	139	140	141	142
11	143	144	145	146	147	148	149	150	151	152	153	154	155	156
12	157	158	159	160	161	162	163	164	165	166	167	168	169	170
13	171	172	173	174	175	176	177	178	179	180	181	182	183	184
14	185	186	187	188	189	190	191	192	193	194	195	196	197	198
15	199	200	201	202	203	204	205	206	207	208	209	210	211	212
16	213	214	215	216	217	218	219	220	221	222	223	224	225	226
17	227	228	229	230	231	232	233	234	235	236	237	238	239	240
18	241	242	243	244	245	246	247	248	249	250	251	252	253	254
19	255	256	257	258	259	260	261	262	263	264	265	266	267	268
20	269	270	271	272	273	274	275	276	277	278	279	280	281	282
21	283	284	285	286	287	288	289	290	291	292	293	294	295	296
22	297	298	299	300	301	302	303	304	305	306	307	308	309	310
23	311	312	313	314	315	316	317	318	319	320	321	322	323	324
24	325	326	327	328	329	330	331	332	333	334	335	336	337	338
25	339	340	341	342	343	344	345	346	347	348	349	350	351	352
26	353	354	355	356	357	358	359	360	361	362	363	364	365	366

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three members of the series of each column. If the m/z numbers of the table are extended beyond m/z 366, the possible Z values will also need to be extended, in units of -14.

The carbon number column at the extreme left of the table indicates the minimum number of carbon atoms that can be used to build a plausible formula for hydrocarbons in that row. The combination of carbon number and Z value makes possible a quick assessment of possible elemental formulas for any given mass value.

2. Probable Elemental Composition Assignments

The range of possible elemental composition assignments for any major peak in a mass spectrum may be narrowed to not more than a few possibilities through use of Table 3. The logic of the Z table alone illustrates the small number of elemental formulas that are possible at any given mass providing the range of possible heteroelement inclusions is restricted. The possible hydrocarbon-only compositions for each amu range from 1 at the lowest m/z values to 5 at the highest m/z on the table (366). Approximately the same number of possible compositions will be possible for each added heteroelement, that is, 1 to 5 for C_yH_xN composition, 1 to 5 for $C_yH_xN_2$, and so on. Although the total number of possible compositions is surprisingly limited, it is still too large for most analysis purposes. However, it will frequently be found that the range of possible elements may be restrictable on the basis of other considerations. In such cases, elemental assignment may be sufficiently narrowed to be effective as an analysis aid.

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TOP

The possible compositions for a peak of m/z 231 are calculated as an example of the procedure.

(1) Hydrocarbon composition only:

(a) The carbon number column shows that the minimum number of carbons is 17. The first Z value for the column is -7. The lowest carbon number composition is then:

$$C_{17}H_{2(17)-7} = C_{17}H_{27} = 231$$

(b) For the next higher carbon number (18), the next Z value should be used:

$$C_{18}H_{2(18)-21} = C_{18}H_{15} = 231$$

(c) The next carbon number (19), has a Z value of -35:

$$C_{19}H_{2(19)-35} = C_{19}H_{3} = 231$$

Case (C) can be dismissed from most considerations, as it is an implausible composition. Thus $C_{17}H_{27}$ and $C_{18}H_{15}$ are the only possible relaistic hydrocarbon compositions for m/z 231.

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(2) For hydrocarbon and one nitrogen:

(1) The first step is to subtract out the mass value of the heteroelement(s) and then proceed as with hydrocarbons. In this example, for one nitrogen, m/z 231-14 = 217. Then, the minimum C number and corresponding Z value for m/z 217 is taken from Table 3:

$$C_{16}H_{2(16)-7}N - C_{16}H_{25}N = 231$$

Page Numbering

USE THIS SHEET FOR REPORT TYPING 8-1/2 x 11

TOP

and, (b) $C_{17}H_{2(17)-21}N = C_{17}H_{13}N = 231$

also, (c) $C_{18}H_{2(18)-35}N = C_{18}H_{1}N = 231$

The last composition may be disregarded as unrealistic.

(3) For hydrocarbon and two nitrogens:

(a) m/z 231-28 = 203; then the minimum C number and corresponding Z value for m/z 203 is taken from Table 3:

$C_{15}H_{2(15)-7}N_2 = C_{15}H_{23}N_2 = 231$, etc.

(4) For hydrocarbon and oxygen:

(a) m/z 231-16 = 215; then

$C_{16}H_{2(16)-9}O = C_{16}H_{23}O = 231$

and, $C_{17}H_{2(17)-23}O = C_{17}H_{11}O = 231$, etc.

For m/z 231, the complete list of possible compositions that contain the elements C, H, N, and O, with no more than two heteroelements in any one composition, is as follows:

$C_{17}H_{27}$
$C_{18}H_{15}$
$C_{16}H_{25}N$
$C_{17}H_{13}N$
$C_{15}H_{23}N_2$
$C_{16}H_{11}N_2$
$C_{16}H_{23}O$
$C_{17}H_{11}O$
$C_{15}H_{19}O_2$
$C_{16}H_{7}O_2$
$C_{15}H_{21}NO$

10 Lines to End of Typing Area

For Page Numbering

USE THIS SHEET FOR REPORT TYPING 8-1/2 x 11

TOP

In most situations, at least half and quite possibly more of these possible compositions could be elminated on the basis of other spectral considerations.

3. The Chemical Class Tables

The Z table also offers sorting and correlation properties that are usable from LRMS data, in much the same manner as are the rings plus double bonds (R+DB) values from elemental composition assignments in HRMS data (Stauffer, *et al*).[32] Each column of the Z table can, for molecular ions, be identified with a relatively small group of chemical classes. A representative listing of chemical class for each of the columns of Table 3 is given in Tables 4 and 5, for molecular ions only. Table 4 is primarily for those classes that are expected to appear in the early LC fractions; Table 5 is for those more likely to appear in the late fractions. It needs to be emphasized that these are representative listings that should not be viewed as complete or exhaustive. Most of the listings are also for a single functional group; combinations thereof will need to be calculated for determination of the proper column position.

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Table 3, 4, and 5 will generally be most effective when used together on an interactive basis. When seeking to learn some possible chemical classes for specific mass spectral features, one would first use the Z table to find the column designation of the mass peaks, and then the chemical class tables for the classes correlatable to that column. Conversely, one may seek specific chemical classes in the mass spectra by

For Page Numbering

279-200M

USE THIS SHEET FOR REPORT TYPING 8 1/2 X 11

TOP

TABLE 4

CHEMICAL CLASS BY Z VALUE, LC FRACTION 1 TO 4

+2[1]	0	-2	-4	-6	-8	-10
Alkanes	Monoalkenes	Dienes	Trienes	Benzenes(78)	Styrenes(104)	Indenes(116)
Naphthalenes(128)[2]	Cycloalkanes(84)	Monoalkynes	3 ring PAH(178)	Benzthiophene(134)	Indanes(118)	4 ring PAH(228)
Dibenzthiophenes(184)	Thiophenes(84)	Cycloalkenes(82)	6 ring PAH(276)	6 ring PAH(302)	4 ring PAH(202)	7 ring PAH(326)
5 ring PAH(226)	3 ring PAH(154)	Fluorobenzenes(96)		6 ring PAH(316)	4 ring PAH(216)	Sulfur(256)
7 ring PAH(352)	5 ring PAH(252)	3 ring PAH(152)			7 ring PAH(300)	
7 ring PAH(366)	5 ring PAH(266)	3 ring PAH(166)			6 ring PAH(328)	
	8 ring PAH(350)	5 ring PAH(278)				

[1] And coinciding Z values: +2, -12, -26 (similarily throughout the table)

[2] Lowest molecular weight of class

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For Page Numbering

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TOP

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TABLE 5

CHEMICAL CLASS BY Z VALUE, LC FRACTION 4 TO 7

+2[1]	+1	0	-1	-2	-3	-4
Ketones	5 ring N[2](267)	Thiophenes(84)	Alkane-nitriles	Furans(68)	Pyrroles(67)	Pyrazines(80)
Aldehydes		Cyclohexanones(98)	Triazoles(69)	Pyrazoles(68)	Pyridinols(95)	Phenols(94)
Biphenylols(170)[3]		Dibenzofurans(168)	Naphthalene-amines(153)	Pyranones(96)	Pyridinones(95)	Quinones(108)
Dibenzthiophenes(184)		Benzophenones(182)	Carbazoles(167)	Thiophenols(110)	Pyridine-Amines(95)	Phenyl Alkyl-Ethers(108)
			5 ring N(279)	Alkyl Ether-Phenols(124)	Amino Phenols(109)	Benzene-diamines(108)
				Fluorenone(180)	Acridines(179)	
				Anthraquinones(208)		

-5	-6	-7	-8	-9	-10	-11
Pyridines(79)	Benzaldehydes(106)	4 ring N(203)	Mercapto Alkanes	Alkyl Amino Alcohols	Alcohols	Alkyl Amines
Anilines(93)	Benzthiophenes(134)	Benzocarbazoles(217)	Alkyl Diols	Nitro Alkanes	Fatty Acids	Alkyl Amides
6 ring N(303)		7 ring N(301)	Alkyl Sulfides	Benzonitriles(103)	Fatty Acid Esters	Quinolines(129)
			Thio ureas(76)	4 ring N(229)	Alkyl Ethers	
			Benzofluoranones (230)		Naphthols(144)	

[1] And coinciding Z values: +2, -12, -26 (similarily throughout the table)

[2] 5 ring N = 5 ring heterocyclic nitrogen

[3] Lowest molecular weight of class

For Page Numbering

USE THIS SHEET FOR REPORT TYPING 8 1/2 X 11

TOP

first using the chemical class tables to find the appropriate Z table column, and then the Z table for a listing of the ions of that class.

4. Chemical Class Parent-Fragment Relationships

Tables 3, 4, and 5 are usable not only for molecular ions but for many fragment ions as well. In order to most effectively make use of the fragment ions, it is helpful to first illustrate some generalized features of mass spectral fragmentation.

Molecular ion fragment ion probabilities for the mass spectra of 12 different classes of compounds are shown in Figure 8. The plots for each compound category display the relative abundance (bar height) of ions at each value. The shaded bar in the graph represents the molecular ion and the open bars represent the fragment ions. Figure 8, which has been redrawn from Crawford and Morrison,[33] was developed from the mass spectra of representative compounds for each class. The spectra of up to 36 compounds per class were each summed over 14 amu intervals, and the values of the individual members of each class averaged, to produce the plots shown. The plot for each compound class may be considered as a generalized spectrum for that class. Possible m/z numerical values for any bar of Figure 8 may be obtained by reference to Table 3. In the case of alkanes, for example, the most probable ions are those fragments for which Z=+1. The major ions of an alkane spectrum can (from Table 3) thus be expected at (43), 57, 71, 85,... Alkenes will have their most abundant ions in the Z=-1 column: 55, 69, 83,... The height of the bar for each Z value of a compound class is indicative

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For Page Numbering

1279 200M

USE THIS SHEET FOR REPORT TYPING 8-1/2 x 11

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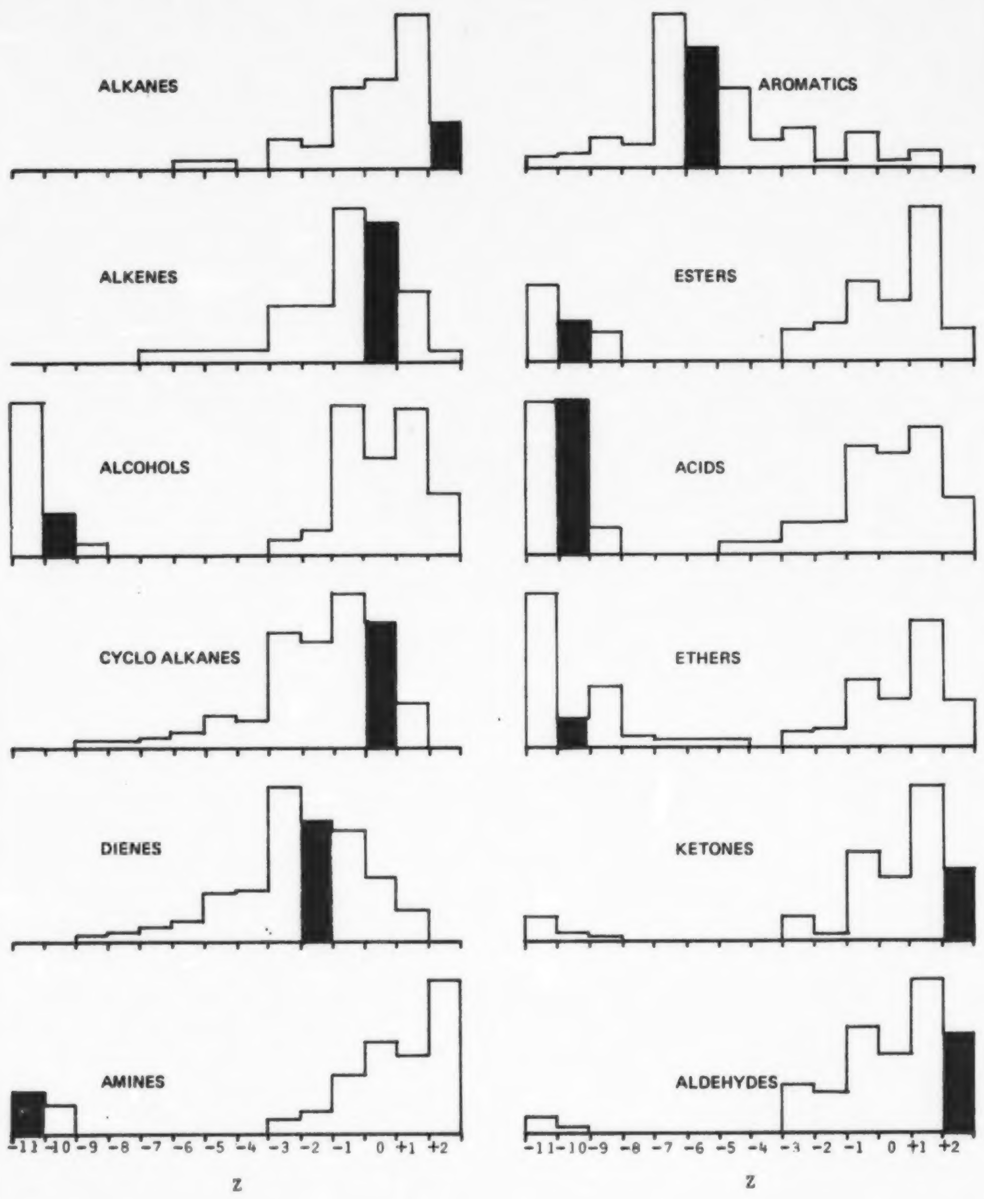

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FIGURE 8 CONDENSED SPECTRAL PATTERNS

For Page Numbering

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of the probable combined intensity of all m/z values that have that Z value in a mass spectrum of a compound of that class.

The height of the shaded bar is not necessarily indicative of the intensity of the molecular ion, but of all m/z values in the spectrum that have the same Z value as the molecular ion. However, since fragmentations to the same Z value as the molecular ion are very uncommon in mass spectra, the contribution to the total molecular ion Z value from ions other than the molecular ion, should be minimal. Thus the height of the shaded bar may be taken as a rough indication of the relative molecular ion intensity to be expected in spectra of members of the class.

It can be seen from Figure 8 that fragment ions show some specificity for Z value, and consequently for chemical class. The specificity for the fragments is perhaps two to five times lower than that of the parent ions, but still high enough to be of potential value. For example, if the major ions of a mass spectrum fall into the -11 Z column, then alkanes, alcohols, and cycloalkanes are plausible. Figure 8 may also be used in the other direction to indicate probable spectral distributions of a suspected class.

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The most generally applicable rule of parent-fragment relationships that can be drawn from Figure 8 is that major aliphatic fragment ions will be located in the column immediately to the left of the molecular ion. This rule is particularly valuable when using Table 3 for species with large alkyl substituents: alkyl phenols, alkyl benzenes, etc. Fragmentation of other than alkyl functional units will show greater column shifts from the parent ion. Loss of water will shift the remaining lower mass fragments four columns to the left; loss of a phenyl group will shift the remaining fragments seven columns to the left, and so on. The Z table can be used to correlate not only primary fragmentations, but subsequent ones as well.

5. Detection of Related Species or Classes

An important element for identification of mixture components is often obtained through observation of homologous series and/or related classes. The correlative properties of the Z table can be very useful both for observing relationships between spectral peaks, and for predicting where such relationships might occur. The ease with which CH_2 based homologous series can be observed is an obvious example. Use of the supplementary Tables 4 and 5, and Figure 8 can expand this potential.

The chemical class tables will not only identify a set of possible classes for MS peaks, but can also suggest additional candidate classes for consideration, and direct the analyst to their spectral location. Figure 8 will be found helpful when the classes under consideration do not show strong molecular ions, for both directions of correlation.

6. Tracing of Alkylated Species or Classes to the Base Ion

Effluent samples frequently contain species that are alkylated to a considerable degree. This can be confusing to the analyst, as reference spectrum information is often not available for alkyl substituents beyond methyl or ethyl groups. When one can (1) observe that an alkyl series is present, and (2) trace that series to the base or nuclear ion, the identification of the class is facilitated. The alkylated PAH's are a case in point. In this specific case there would be no class characteristic base ion from a pure compound, say a C_3 substituted anthracene. But an effluent sample that contained a significant C_3 alkyl anthracene/phenanthrene would almost certainly also contain a C_2 and C_1 substituted anthracene/phenanthrene, and the anthracene/phenanthrene peak also. Consequently in such a mixture the series m/z 178, 192, 206, 220 would appear, though it may be necessary to examine data over a range of probe temperatures in order to observe it. In this particular example the correlation of a homologous series of molecular ions is used to confirm the identification.

One may just as frequently need to trace a series of fragment ions. The fragment ions of a homologous series are usually common to most if not all of the species of the series, and so they add together. This may result in a series of prominent ions for which the molecular ions are disproportionately small. A series of significant ions in the -5 column of the Z table immediately suggests that there could be a molecular ion or series of molecular ions in the -4 column. If a series of alkylated phenols is present with a molecular weight range of, say, 220 to 262, then the fragment ions of each of the homologous phenols will add together to produde a fragment series that is unexpectedly intense as compared to the molecular peaks. When such a series of fragment ions is observed, reference to the Z table can quickly show where the molecular ions might be. Their detection and correlation to the fragment ions is thus considerably facilitated.

The Z table, along with the chemical class tables, may be used for intra-class or species correlations as well as for inter-class or species comparisons. A homologous series of peaks, for example, has a high probability of intra-relationship. Such a series can be associated with either the molecular ion or other class indicative ion by a suitable column shift. The most likely column shift may be first estimated through reference to Figure 8. Since this figure contains a very limited range of chemical classes, it may be necessary to make inference to other classes. The chemical class Tables 4 and 5 can be helpful in this regard.

C. Polycyclic Organic Materials

The tables and figures of this section are provided to assist the analyst in the recognition and reporting of polycyclic organic materials. The principal development has been given to the PAH class of compounds; the heterocyclic nitrogen, sulfur, and oxygen analog species are treated as derivative. For simplicity, only completely fused, fully aromatic species are included in the compilations.

1. The PAH Charts

The mass spectra of the PAH species are simple and comparatively easy to interpret. With an intense molecular ion, minimal fragmentation, and characteristic molecular weights, they are generally easy to assign to chemical category and molecular weight range. But there are complicating factors in their evaluation from industrial and environmental sources. The mixtures are often quite complex, with components derived from many base nuclei, and extensive alkylation of the base nuclei. Alkyl substituents may range up to C_{10} and even higher.[34]

The many isomers possible for a given composition are not distinquisable by LRMS, as the spectra are essentially identical. The number of possible isomers goes up very quickly with increasing molecular weight. The early members of the series in which no more than two carbon atoms are shared between rings, (ortho fused systems) has the following possibilities:

M/Z	No. of Rings	Composition	Possible Isomers
128	2	$C_{10}H_8$	2
178	3	$C_{14}H_{10}$	2
228	4	$C_{18}H_{12}$	5
278	5	$C_{22}H_{14}$	12

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TOP

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It is reasonable to assume that in complex mixtures several of the possible isomers may be present, and since there is no rational way to decide which they might be, it could be considered inaccurate to list any. On the other hand, molecular weight, composition, and in almost all cases the number of rings will all be unambiguously determined by direct probe LRMS. These should be reported, along with the intensity to be assigned to that peak. Along with that information it can be helpful to list specific possible compounds as representative examples. Species "identifications" of this sort should be shown in parenthesis to indicate that they are representative rather than specific, with an indication that they are an example, as in the following illustration:

Identified Components	MW	Composition
4 ring PAH (e.g., chrysene, benzanthracenes)	228	$C_{18}H_{12}$

The molecular weight of unsubstituted PAH species serves to unambigously identify the elemental composition of the molecular ion, and the number of rings for that composition. This information is listed for ready reference in Table 6 where for convenience it is ordered by m/z value. It is shown structurally ordered in Table 7 and in Figure 9. Information is given for species with up to ten rings, as this is typical of the range that may be observed by LRMS via the direct insertion probe. Occasionally higher homologs may be observed; in such cases, the table can easily be extended. Only one of the possible isomers for each composition is shown in Figure 9. The number of rings will remain the same for all of the possible isomers.

For Page Numbering

TABLE 6

PAH NUCLEUS LIST

M/Z	Rings	Composition	R&DB[1]	Z
66	1	C_5H_6	3	-4
78	1	C_6H_6	4	-6
116	2	C_9H_8	6	-10
128	2	$C_{10}H_8$	7	-12
152	3	$C_{12}H_8$	9	-16
166	3	$C_{13}H_{10}$	9	-16
178	3	$C_{14}H_{10}$	10	-18
202	4	$C_{16}H_{10}$	12	-22
216	4	$C_{17}H_{12}$	12	-22
226	5	$C_{18}H_{10}$	14	-26
228	4	$C_{18}H_{12}$	13	-24
252	5	$C_{20}H_{12}$	15	-28
266	5	$C_{21}H_{14}$	15	-28
276	6	$C_{22}H_{12}$	17	-32
278	5	$C_{22}H_{14}$	16	-30
300	7	$C_{24}H_{12}$	19	-36
302	6	$C_{24}H_{14}$	18	-34
316	6	$C_{25}H_{16}$	18	-34
326	7	$C_{26}H_{14}$	20	-40
328	6	$C_{26}H_{16}$	19	-36
350	8	$C_{28}H_{14}$	22	-42
352	7	$C_{28}H_{16}$	21	-40
366	7	$C_{29}H_{18}$	21	-40
374	9	$C_{30}H_{14}$	24	-46
376	8	$C_{30}H_{16}$	23	-44

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TABLE 6 (Continued)

PAH NUCLEUS LIST

M/Z	Rings	Composition	R&DB	Z
378	7	$C_{30}H_{18}$	22	-42
400	9	$C_{32}H_{16}$	25	-48
402	8	$C_{32}H_{18}$	24	-46
416	8	$C_{33}H_{20}$	24	-46
424	10	$C_{34}H_{16}$	27	-52
426	9	$C_{34}H_{18}$	26	-50
428	8	$C_{34}H_{20}$	25	-48
450	10	$C_{36}H_{18}$	28	-54
452	9	$C_{36}H_{20}$	27	-52
466	9	$C_{37}H_{22}$	27	-52
474	11	$C_{38}H_{18}$	30	-58
476	10	$C_{38}H_{20}$	29	-56
478	9	$C_{38}H_{22}$	28	-54
500	11	$C_{40}H_{20}$	31	-60

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[1] R&DB = Rings and double bonds

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TABLE 7

PAH COMPOSITION, STRUCTURALLY ORDERED

1 Ring	2 Rings	3 Rings	4 Rings	5 Rings	6 Rings	7 Rings	8 Rings	9 Rings	10 Rings
$\frac{78}{C_6H_6}$[1]	$\frac{128}{C_{10}H_8}$	$\frac{178}{C_{14}H_{10}}$	$\frac{228}{C_{18}H_{12}}$	$\frac{278}{C_{22}H_{14}}$	$\frac{328}{C_{26}H_{16}}$	$\frac{378}{C_{30}H_{18}}$	$\frac{428}{C_{34}H_{20}}$	$\frac{478}{C_{38}H_{22}}$	$\frac{528}{C_{42}H_{26}}$
$\frac{66}{C_5H_6}$	$\frac{116}{C_9H_8}$	$\frac{166}{C_{13}H_{10}}$	$\frac{216}{C_{17}H_{12}}$	$\frac{266}{C_{21}H_{14}}$	$\frac{316}{C_{25}H_{16}}$	$\frac{366}{C_{29}H_{18}}$	$\frac{416}{C_{33}H_{20}}$	$\frac{466}{C_{37}H_{22}}$	$\frac{516}{C_{41}H_{24}}$
		$\frac{152}{C_{12}H_8}$	$\frac{202}{C_{16}H_{10}}$	$\frac{252}{C_{20}H_{12}}$	$\frac{302}{C_{24}H_{14}}$	$\frac{352}{C_{28}H_{16}}$	$\frac{402}{C_{32}H_{18}}$	$\frac{452}{C_{36}H_{20}}$	$\frac{502}{C_{40}H_{22}}$
				$\frac{226}{C_{18}H_{10}}$	$\frac{276}{C_{22}H_{12}}$	$\frac{326}{C_{26}H_{14}}$	$\frac{376}{C_{30}H_{16}}$	$\frac{426}{C_{34}H_{18}}$	$\frac{476}{C_{38}H_{20}}$
						$\frac{300}{C_{24}H_{12}}$	$\frac{350}{C_{28}H_{14}}$	$\frac{400}{C_{32}H_{16}}$	$\frac{450}{C_{36}H_{18}}$
								$\frac{374}{C_{30}H_{14}}$	$\frac{424}{C_{34}H_{16}}$

[1] mw of composition

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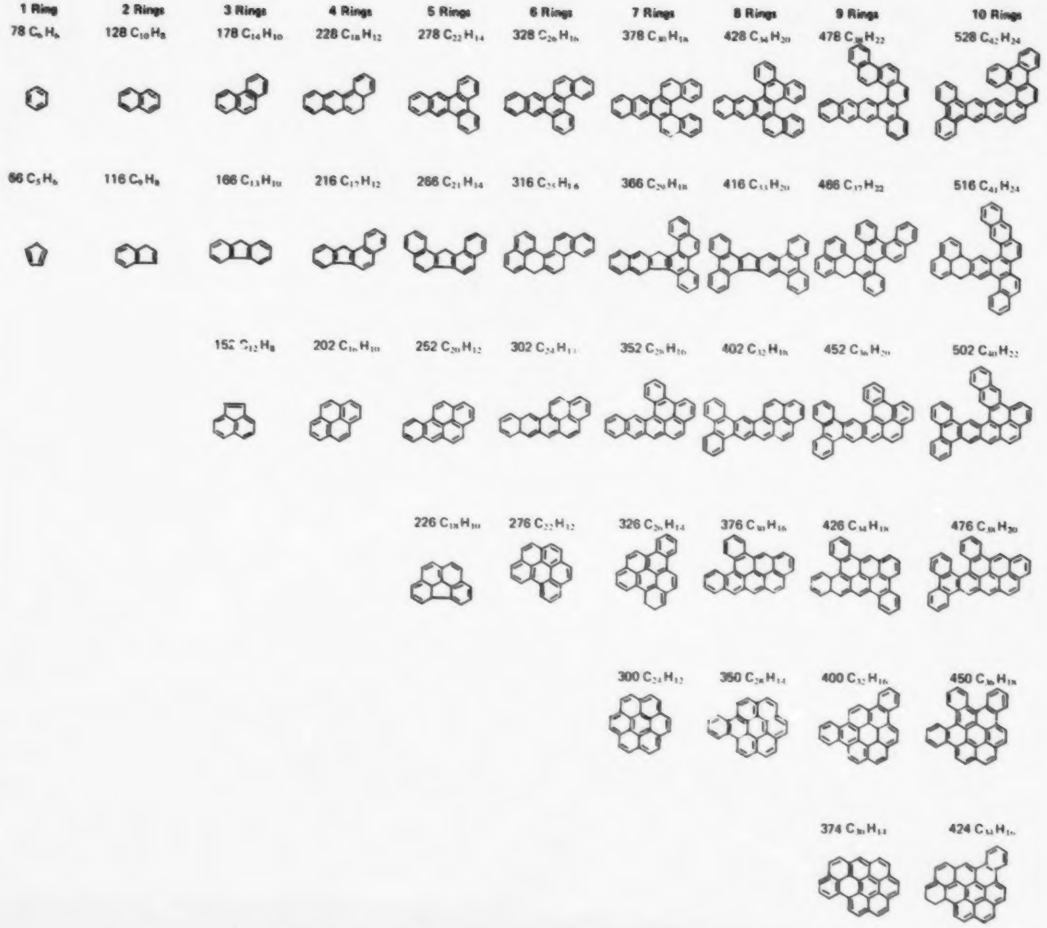
1 Ring
78 C_6H_6
2 Rings
128 $C_{10}H_8$
3 Rings
178 $C_{14}H_{10}$
4 Rings
228 $C_{18}H_{12}$
5 Rings
278 $C_{22}H_{14}$
6 Rings
328 $C_{26}H_{16}$
7 Rings
378 $C_{30}H_{18}$
8 Rings
428 $C_{34}H_{20}$
9 Rings
478 $C_{38}H_{22}$
10 Rings
528 $C_{42}H_{24}$
66 C_5H_6
116 C_9H_8
166 $C_{13}H_{10}$
216 $C_{17}H_{12}$
266 $C_{21}H_{14}$
316 $C_{25}H_{16}$
366 $C_{29}H_{18}$
416 $C_{33}H_{20}$
466 $C_{37}H_{22}$
516 $C_{41}H_{24}$
152 $C_{12}H_8$
202 $C_{16}H_{10}$
252 $C_{20}H_{12}$
302 $C_{24}H_{14}$
352 $C_{28}H_{16}$
402 $C_{32}H_{18}$
452 $C_{36}H_{20}$
502 $C_{40}H_{22}$
226 $C_{18}H_{10}$
276 $C_{22}H_{12}$
326 $C_{26}H_{14}$
376 $C_{30}H_{16}$
426 $C_{34}H_{18}$
476 $C_{38}H_{20}$
300 $C_{24}H_{12}$
350 $C_{28}H_{14}$
400 $C_{32}H_{16}$
450 $C_{36}H_{18}$
374 $C_{30}H_{14}$
424 $C_{34}H_{16}$

FIGURE 9 PAH STRUCTURAL SERIES

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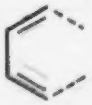
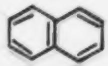
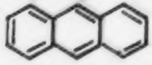
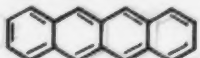
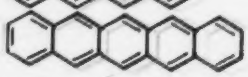
The information presented in the PAH charts was developed in the following manner. The basic concept is that all of the common PAH species can be viewed as members of a limited set of series, each of which is homologous in units of C_4H_2, m/z 50:



as shown in Figure 9. Each addition of that unit adds exactly one ring to the structure of the preceeding member of the series, as well as increasing the mw by 50, and the composition by C_4H_2. The first, and lowest molecular weight unit of each series, represents one degree greater of fusion or condensation, than did the first member of the proceeding series. The same degree of fusion is maintained throughout a series.

The first row of Table 7 and Figure 9 is the ortho fused series:

M/Z		Composition	No. of Rings
128		$C_{10}H_8$	2
178		$C_{14}H_{10}$	3
228		$C_{18}H_{12}$	4
278		$C_{22}H_{14}$	5

etc.

This series has the highest molecular weight for any given number of rings, as it yields species with the lowest degree of fusion possible for a fully fused system.

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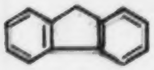
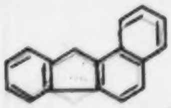
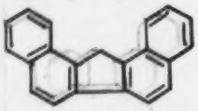
A similar series is the one which begins with flourene:

M/Z		Composition	No. of Rings
166		$C_{13}H_{10}$	3
216		$C_{17}H_{12}$	4
266		$C_{21}H_{14}$	5

etc.

This series is predominantly ortho fused as well, with a few ortho-peri fused species. The species of this series each contain one saturated carbon atom. If the saturated carbon atom is in a five membered ring, the species will be ortho fused. If the saturated carbon is part of a six membered ring, that ring will be in a peri fused configuration. This series gives mass spectra that are somewhat different than other PAH species. They show a substantial M-1 peak that may be nearly as intense as the molecular ion. The series coincides in molecular weight, composition, number of rings, and mass spectra with the methylated acenaphthylene series.

The acenaphthylene series is peri and ortho fused throughout its molecular weight range:

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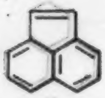
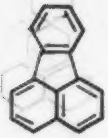
M/Z		Composition	No. of Rings
152		$C_{12}H_8$	3
202		$C_{16}H_{10}$	4
252	etc.	$C_{20}H_{12}$	5
302	etc.		

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Additional series begin at m/z 226, 300, 374, 398 and at higher m/z values. Each succeeding series arises from the next higher degree of condensation, and represents that extent of condensation throughout. The PAH charts are essentially complete to mass 400 for the fully fused species. However, for simplicity the table does not contain any elements which have unfused rings (biphenyl, m/z 154; benzyl napthalene, m/z 218; etc.) nor does it show hydrogenated or alkylated species. These materials are in fact frequently encountered in effluent samples, but their composition will usually be inferrable either directly from the PAH charts, or in conjunction with Table 3.

2. The Aza Arenes

The aza arene equivalents of the PAH compositions are listed by molecular weight in Table 8 to m/z 304. Since nitrogen can readily substitute for carbon in the rings without altering the pattern of the rings,[35] it is not necessary to show separate charts for these species. A special caution for the analyst is needed in regard to the amine substituted PAH species. These are not included in the tables presented

For Page Numbering

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TABLE 8

AZA-PAH NUCLEUS LIST (N_1,N_2)

M/Z	Rings	Composition	R&DB[1]	Z[2]
67	1	C_4H_5N	3	-3N
68	1	$C_3H_4N_2$	3	$-2N_2$
79	1	C_5H_5N	4	-5N
80	1	$C_4H_4N_2$	4	$-4N_2$
117	2	C_8H_7N	6	-9N
118	2	$C_7H_6N_2$	6	$-8N_2$
129	2	C_9H_7N	7	-11N
130	2	$C_8H_6N_2$	7	$-10N_2$
153	3	$C_{11}H_7N$	9	-15N
154	3	$C_{10}H_6N_2$	9	$-14N_2$
167	3	$C_{12}H_9N$	9	-15N
168	3	$C_{11}H_8N_2$	9	$-14N_2$
179	3	$C_{13}H_9N$	10	-17N
180	3	$C_{12}H_8N_2$	10	$-16N_2$
203	4	$C_{15}H_9N$	12	-21N
204	4	$C_{14}H_8N_2$	12	$-20N_2$
217	4	$C_{16}H_{11}N$	12	-21N
218	4	$C_{15}H_{10}N_2$	12	$-20N_2$
227	5	$C_{17}H_9N$	14	-25N
228	5	$C_{16}H_8N_2$	14	$-24N_2$
229	4	$C_{17}H_{11}N$	13	-23N
230	4	$C_{16}H_{10}N_2$	13	$-22N_2$
253	5	$C_{19}H_{11}N$	15	-27N
254	5	$C_{18}H_{10}N_2$	15	$-26N_2$
267	5	$C_{20}H_{13}N$	15	-27N
268	5	$C_{19}H_{12}N_2$	15	$-26N_2$

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TABLE 8 (Continued)

AZA-PAH NUCLEUS LIST (N_1,N_2)

M/Z	Rings	Composition	R&DB	Z
277	6	$C_{21}H_{11}N$	17	$-31N$
278	6	$C_{20}H_{10}N_2$	17	$-30N_2$
279	5	$C_{21}H_{13}N$	16	$-29N$
280	5	$C_{20}H_{12}N_2$	16	$-28N_2$
301	7	$C_{23}H_{11}N$	19	$-35N$
302	7	$C_{22}H_{10}N_2$	19	$-34N_2$
303	6	$C_{23}H_{13}N$	18	$-33N$
304	6	$C_{22}H_{12}N_2$	18	$-32N_2$

[1] R&DB = Rings and double bonds

[2] The numerical portion of this column is equal to the Z value of the hydrocarbon portion of the molecule; the heteroelement is also listed as a reminder of its presence in the molecule.

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For Page Numbering

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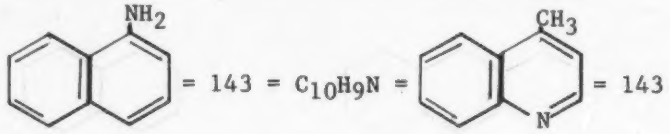
here, as they are substituents on the otherwise charted PAH or heteroelement nuclear species. It is not possible on the basis of molecular weight or elemental composition to distinguish between an aromatic amine and a methylated heterocyclic nitrogen species:



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Within the Level scheme the best information for resolution of this difficulty is probably obtainable through close cooperation with the IR analyst.

3. The Oxygen and Sulfur Polycyclics

The potential for complexity from oxygen or sulfur substituents in or on the aromatic rings is significantly greater than that of the PAH or aza arene species. Oxygen may be found in the ring, attached in a ketone function, in an ether or hydroxy substituent, or in any combination of these features. Sulfur may be equally complex, as it can take any of the positions in a polycyclic system that are available to oxygen, plus some of its own. In addition, combinations of both heteroelements can occur in the same species.

It is clear that an attempt to make an exhaustive chart for all of the possible combinations would result in something so complex as to be of limited use. Furthermore it would probably contain many species that would very rarely, if ever, be observed.

For Page Numbering

It is for these reasons that Figures 10 and 11 contain only a representative sampling of the oxygenated and sulfur polycyclics, with a few examples of mixed species. These tables illustrate some of the kinds of materials that may be encountered, but they are far from being a complete catalog of the possible species.

An attempt has been made to give a reasonably complete illustration of the ways in which the heteroelements may become incorporated into the molecule, and of the base units on which a series can develop. An additional possible form not shown here is the endoperoxide reported by Fox and Olive.[36] (This form may be very difficult to identify by LRMS). As each unit in any series grows more complex, comparably more isomeric configurations become possible for the base units. Only one possibility is shown for each composition.

All of the base units shown may also be encountered in alkylated or otherwise substituted forms. The substituents may contain additional heteroelement functions. Here again, Table 3 may be of help for correlative purposes.

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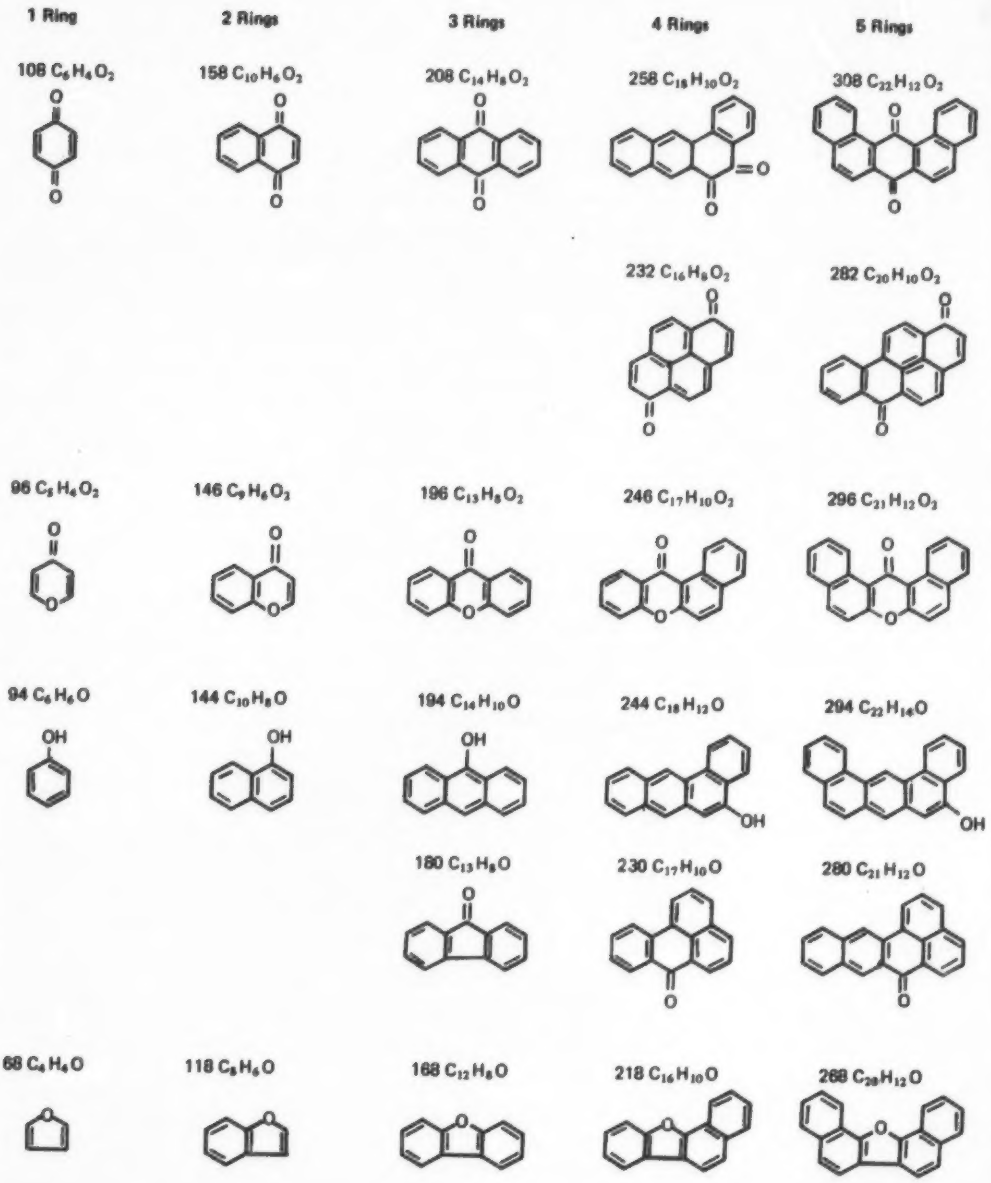
1 Ring
2 Rings
3 Rings
4 Rings
5 Rings
108 $C_6H_4O_2$
158 $C_{10}H_6O_2$
208 $C_{14}H_8O_2$
258 $C_{18}H_{10}O_2$
308 $C_{22}H_{12}O_2$
232 $C_{16}H_8O_2$
282 $C_{20}H_{10}O_2$
96 $C_5H_4O_2$
146 $C_9H_6O_2$
196 $C_{13}H_8O_2$
246 $C_{17}H_{10}O_2$
296 $C_{21}H_{12}O_2$
94 C_6H_6O
144 $C_{10}H_8O$
194 $C_{14}H_{10}O$
244 $C_{18}H_{12}O$
294 $C_{22}H_{14}O$
180 $C_{13}H_8O$
230 $C_{17}H_{10}O$
280 $C_{21}H_{12}O$
68 C_4H_4O
118 C_8H_6O
168 $C_{12}H_8O$
218 $C_{16}H_{10}O$
268 $C_{20}H_{12}O$
O
OH

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FIGURE 10 REPRESENTATIVE OXYGENATED POLYCYCLICS

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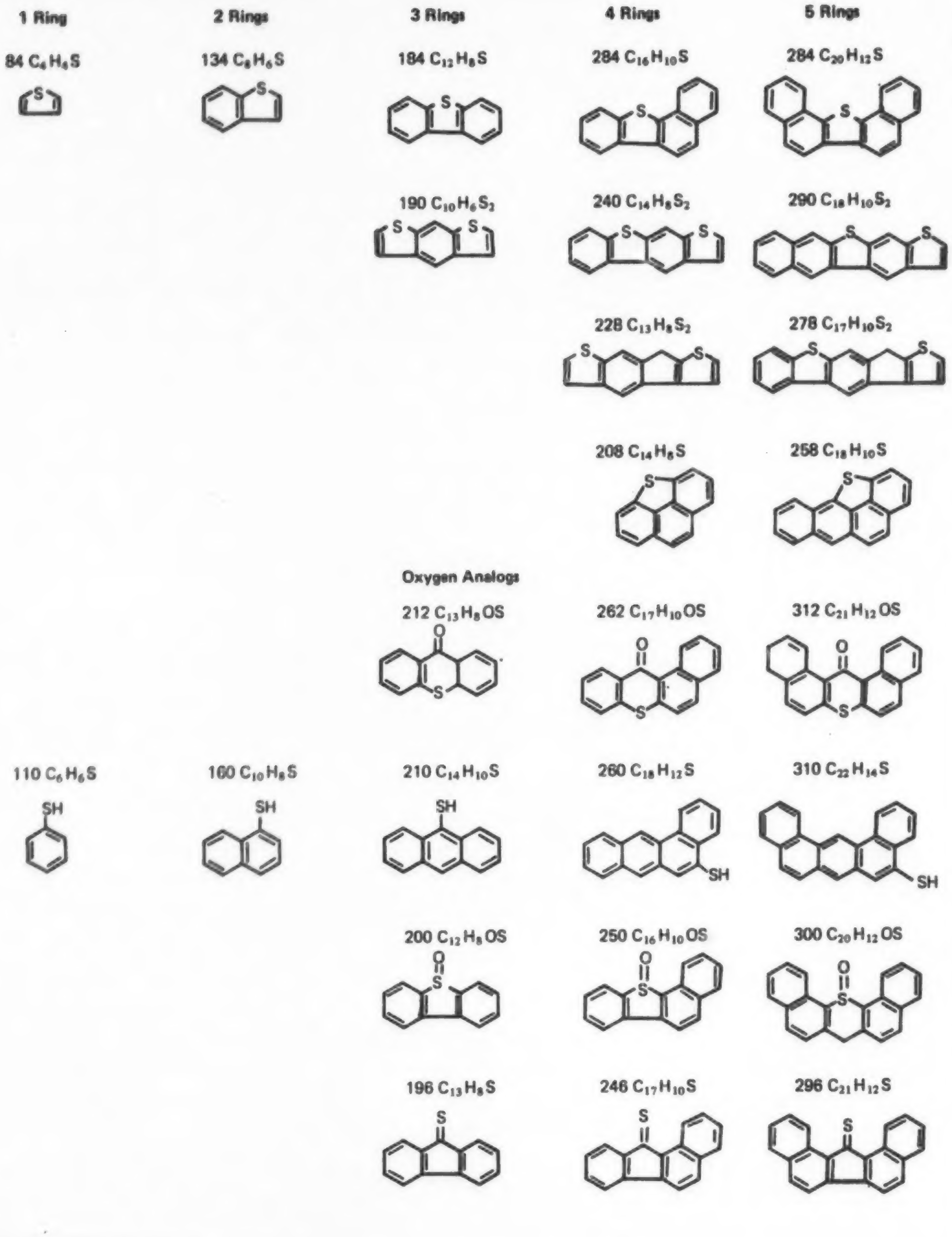
1 Ring
2 Rings
3 Rings
4 Rings
5 Rings
84 C_4H_4S
134 C_8H_6S
184 $C_{12}H_8S$
284 $C_{16}H_{10}S$
284 $C_{20}H_{12}S$
190 $C_{10}H_6S_2$
240 $C_{14}H_8S_2$
290 $C_{18}H_{10}S_2$
228 $C_{13}H_8S_2$
278 $C_{17}H_{10}S_2$
208 $C_{14}H_8S$
258 $C_{18}H_{10}S$
Oxygen Analogs
212 $C_{13}H_8OS$
262 $C_{17}H_{10}OS$
312 $C_{21}H_{12}OS$
110 C_6H_6S
160 $C_{10}H_8S$
210 $C_{14}H_{10}S$
260 $C_{18}H_{12}S$
310 $C_{22}H_{14}S$
200 $C_{12}H_8OS$
250 $C_{16}H_{10}OS$
300 $C_{20}H_{12}OS$
196 $C_{13}H_8S$
246 $C_{17}H_{10}S$
296 $C_{21}H_{12}S$

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FIGURE 11 REPRESENTATIVE SULFUR AND OXYSULFUR HETEROCYCLICS

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TOP

CHAPTER IV

ANALYSIS EXAMPLES

A. Introduction

There are many different sequences that may be followed in the process of deciphering mixed species spectra. The suitability of one approach when compared to another depends principally on the information that is accessible in the spectra. Just what that will be in any given situation depends not only on the nature of the spectra of the individual components, but also on the way in which the individual spectra superimpose. Important information elements from a given species may be distorted or concealed by the spectra of other components.

In complex cases, an effective approach can be obtained by selecting separate elements from different techniques, and using them in new combinations in response to specific opportunities in the spectra. It is in effect as though one were building a somewhat special technique for each challenging problem. The most important element in this may very well be simply to recognize the usable information elements of a spectrum, or of a set of spectra. It is clear that a prerequisite to such recognition is an awareness of many different ways of utilizing spectral information.

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This chapter presents four examples of spectral interpretation as applied to mixture analysis. The analysis of each spectrum is worked out in some detail in order to illustrate a specific procedure that can

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be used with the information that is present in that example.

B. A Polar Mixture

The first of these examples is shown in Figure 12. It is a single spectrum example from a late LC fraction--it could be from LC5, LC6, or LC7. It is a 70 eV spectrum, taken in the middle temperature range. There is no information available from either earlier or later scans, or from the spectra of adjacent LC fractions. The isotope ratios of the spectrum may be reliable, but they will not be considered as a significant information source. This is simply to say that there is no information available that is not contained in the spectrum of Figure 12, except the knowledge that it is a polar LC fraction sample.

The dominant feature of this example spectrum is that it shows many distinct peaks well above what might be called the "confusion level." It can be guessed that all or at least most of these major peaks should be correlateable to specific components of the mixture, if indeed it is a mixture. There are three possibilities for this spectrum:

Case 1: all of the peaks are from the same species;
Case 2: it is a mixture of many components, with only one major peak from each component;
Case 3: it is a mixture of a few components each or most of which contribute a few major peaks to the spectrum.

The three logically possible cases are also all spectroscopically

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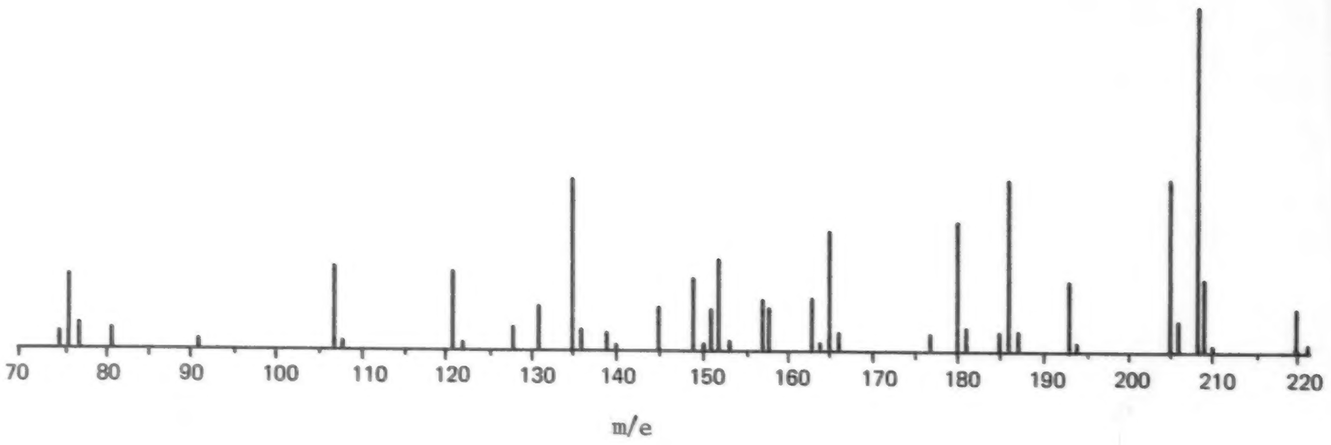

FIGURE 12 EXAMPLE #1: MASS SPECTRUM OF A POLAR LC FRACTION

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possible, though the real chemical probabilities for each are far from equal. Specifically:

Case 1: There are many major peaks that are closer together than any reasonable fragmentation would make likely: m/z 200 and 208; 208 and 205; 193, 186, and 180---and so on. For all of these to come from a single component, a molecule with an extraordinary variety of functional groupings would be needed.

Case 2: If each major peak is to be from a separate component, low molecular weight species such as those at m/z 76 and 107 must be vaporizing simultaneously with those over m/z 200. This is possible, but rather unlikely.

Case 3: This case is left as the most probable, not only by elimination of the other two, but also because a few species with a few peaks each can potentially yield very many different spectra, including this one.

Thus, one begins the analysis with the assumption that several components are involved, but probably not more than a few--and that most of these components will have more than one major peak. The initial step in this case is to try to find two or more peaks that may be associated with the same species. There are several ways that this might be done; in this example one begins by examining the m/z differences between major peaks, using those values to indicate possible neutral losses between the peaks. When a neutral loss suggested by this procedure is plausible, a search of the Eight Peak Index is made based on those two ions. Any spectrum indicated as possible from the Index (within the context of the LC fraction) is then reverse matched against the unknown spectrum.

This sequence of operation involves three stages of selection:

(1) identification or selection of possible neutral losses;
(2) Eight Peak Index major peak match;
(3) reverse match of all significant reference peaks.

These three steps taken in sequence will usually reduce the possibilities to a very few compositions. (The sequence may perhaps be more likely to fail by not identifying any possibilities, than by yielding too many.)

Inasmuch as the high mass ions are generally more compound specific than the lower mass ones, and since fragmentation at high mass values presents fewer possibilities than those at low mass, the initial matching is restricted to the high end of the spectrum. This example uses the range of m/z 220 to m/z 160, but how far one matches down from the highest mass ion is somewhat arbitrary.

Since this sample is from a late LC fraction, nitrogen-containing species are reasonably probable. One must, therefore, include both even and odd mass peaks as possible molecular ions. Small peaks adjacent to an abundant peak are ignored on the first pass, as they have a high probability of being associated with the abundant peak, either as isotopes or as a single H loss.

Table 9 illustrates the first stage of the analysis. All of the possible two major peak associations in the mass range of m/z 220 to m/z 160 are listed, ordered in decreasing mass of the possible molecular ion, subordered in decreasing mass of the associable daughter ion.

The arithmetic difference between the mass values of a potential parent-daughter combination establishes the weight of the neutral loss that would apply for that combination. The probability column lists a value related to the likelihood of that transition actually being true, based on the possible compositions for that weight. Only minimal effort was spent in trying to assess the probability of given neutral losses within the specific spectrum, as compared to mass spectra in general, in order to minimize prejudgment of the results.

Table 9 is now used as a decision table to select those possibilities that are to be considered further. Transitions that are given a probability of 0 or 1 are discarded at this time; those with a probability of 2 or 3 are retained--for clarity they are listed again in Table 10.

Table 10 is now used as a list for searching the Eight Peak Index. The search is based on each of the two indicated mass values. Since in mixed species spectra there is always a possibility of specific ion interferences,

TABLE 9

POSSIBLE PEAK ASSOCIATIONS

Possible Parent Ion	Daughter Ion	Neutral Loss	Probability[1]	Possible Neutral Species
220	208	12	0	-----
220	205	15	3	CH_3
220	193	27	2	CHN
220	186	34	2	H_2S
220	180	40	1	Possible C_3H_4 from Aromatic
220	165	55	2	C_4H_7
220	163	57	2	C_4H_9
208	205	3	0	-----
208	193	15	3	CH_3
208	186	22	0	-----
208	180	28	3	CO, C_2H_4
208	165	43	2	Several Possibilities
208	163	45	2	Carboxylic Acids, Others
205	193	12	0	-----
205	186	19	2	F
205	180	25	0	-----
205	165	40	1	Possible C_3H_4 from Aromatic
205	163	42	2	CH_2CO, C_3H_6
193	186	7	0	-----
193	180	13	0	-----
193	165	28	3	CO, C_2H_4
193	163	30	3	NO
186	180	6	0	-----
186	165	21	0	-----
186	163	23	0	-----
165	163	2	0	-----

---

[1] Probabilities:

0 - Negligible
1 - Low
2 - Moderate
3 - High

TABLE 10

PEAK COMBINATIONS FOR LIBRARY SEARCH

Tentative Parent Ion	Associated Daughter Ion	Indicated Neutral Loss
220	205	CH_3
220	193	CHN
220	186	H_2S
220	165	C_4H_7
220	163	C_4H_9
208	193	CH_3
208	180	CO, C_2H_4
208	165	C_2H_3O, HNCO, C_3H_7...
208	163	COOH, C_2H_5O
205	186	F
205	163	CH_2CO, C_3H_6
193	165	CO, C_2H_4
193	163	NO

more allowance than is usual must be made in comparing intensities. Generally the library is searched for both possible combinations--parent larger than daughter, and vice versa. Even this does not cover all cases, as it is quite possible for other peaks in the spectrum to be more abundant than either of the two peaks being used for the search. If that is the case, the correct identification will be missed with this technique. However, a two peak search based on peaks identified by the method described above will very often include the correct identification; it is a powerful but not infallible approach.

The information obtained from the search with m/z 220 as the parent ion is shown in Table 11--first the forward search to establish all possibilities from the library, then the reverse search to determine if the species suggested by the forward search could be present in the unknown. This procedure leaves as tentative identifications the two possibilities listed at the bottom of the table.

Table 12 is a similar compilation, except that here the reverse search has been performed as soon as the forward search identifies a possibility. This corresponds more closely to what one might do in actual practice, as it represents a significant savings in time and effort. Once again there are two remaining tentative identifications.

The tentative identification of

m/z 220	$C_{15}H_{24}O$	Alkyl phenol (branched)
	$C_{14}H_{20}O_2$	Alkyl benzoquinone

TABLE 11

m/z 220 LIBRARY SEARCH

Parent	Daughter	All Listed Possibilities
220	205	$C_{15}H_{24}O$ Alkyl Phenols, Cedranone
	205 (larger)	$C_{14}H_{20}O_2$ Alkyl Benzoquinones
220	193	No listings for 193 > 220; for 220 > 193: Amino Cyano Phenazine ($C_{13}H_8N_4$), Alkyl Quinoxalines ($C_{15}H_{12}N_2$)
220	186	No listings for 220 > 186 or 186 > 220
220	165	No listings for 165 > 220; for 220 > 165: Phosphorus Triacetate ($C_6H_9O_6P$)
220	163	No listings for 163 > 220 or 220 > 163

Reverse Matching

$C_{15}H_{24}O$ Alkyl Phenols: Acceptable
Cedranone: Fails--missing m/z 123, 109

$C_{14}H_{20}O_2$ Alkyl Benzoquinone: Acceptable, but must have additive interference at m/z 205 and 163.

$C_{13}H_8N_4$ Amino Cyano Phenazine: Fails-missing m/z 110, 192.

$C_{15}H_{12}N_2$ Alkyl Quinoxalines: Fails-missing m/z 89, 90, 219.

$C_6H_9O_6P$ Phosphorus Triacetate: Fails--missing m/z 124.

The Initial Tentative Identifications for m/z 220 are:

$C_{15}H_{24}O$ Alkyl Phenols (Branched)--includes all of m/z 205

$C_{14}H_{20}O_2$ Alkyl Benzoquinones--ID requires another component to be present at m/z 205.

TABLE 12

m/z 208 LIBRARY SEARCH

Parent	Daughter	Possibilities After Reverse Search
208	193	None with 208 > 193. None with 193 > 208, but the 9-Methoxy Anthracene fit is close enough to keep in mind.
208	180	208 > 180: $C_{14}H_8O_2$, Anthraquinone, but must have additive interference at m/z 208--all fragments are in the listed proportions to each other, but only 1/2 as large as would be expected relative to 208. Hexahydropyrene is a good enough fit to keep in consideration, but can be rejected by LC fraction.
208	165	$C_{15}H_{12}O$ Methoxyphenanthrene, can include m/z 193.
208	163	None with m/z 208 > 163; one with m/z 163 > 208.

The Initial Tentative Identifications for m/z 208 are:

$C_{14}H_8O_2$ Anthraquinone, providing there is another component also present at m/z 208.

$C_{15}H_{12}O$ A Methoxy Anthracene possible.

and m/z 208 $C_{14}H_8O_2$ Anthraquinone

$C_{15}H_{12}O$ Methoxy phenanthrene

can be applied to the unknown spectrum through subtraction of the tentative indentifications. The effect is as shown in Figure 13, when applied to the high mass region. Most of the peaks in the high mass region are accounted for by these possibilities, and the appearance of the remaining spectrum has changed substantially. (Strict accounting of intensities has not been followed at this point.)

The dominant features of the remaining spectrum are an m/z 14 difference series of 107, 121, 135, 149, and perhaps 177, which is 28 amu up; and the small group of peaks at m/z 186. Since the mass difference between 186 and 177 is 9, a very unlikely loss, it is probable that there are still at least two components present. If one searches the Eight Peak Index on the basis of the m/z 14 series, using the abundances shown in the spectrum, one finds nonyl phenol ($C_{15}H_{24}O$) and dodecyl phenol ($C_{18}H_{30}O$) as possibilities. There is no way of making a choice between these two via the principal ions--but the 2% to 3% parent ion of dodecyl phenol (m/z 262) should be visible. Since it is not, and since the 4% parent ion of nonyl phenol (m/z 220) could very well be present, that identification is tentatively selected.

The remaining peaks are few: scaled to 186 as the base peak, they are (approximate values):

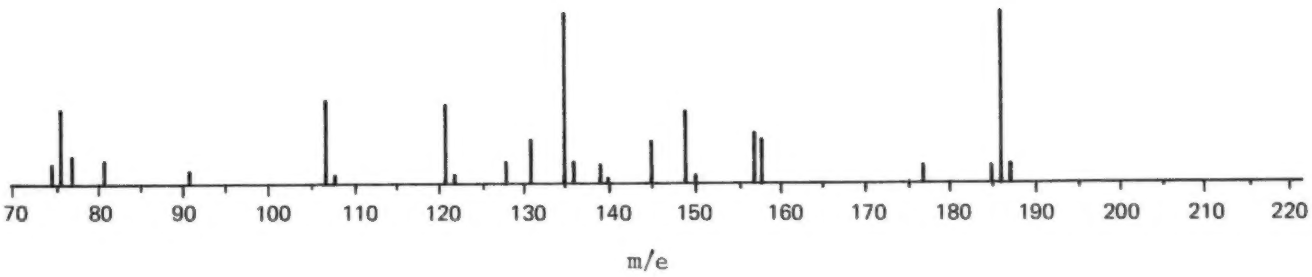

FIGURE 13 SPECTRUM REMAINING AFTER SUBTRACTION OF TENTATIVELY IDENTIFIED COMPONENTS

m/z 186 = 100%
158 = 25
157 = 25
145 = 25
131 = 25
128 = 10
139 = 10
185 = 8
187 = 8

Biphenol ($C_{12}H_{10}O_2$) is the only choice for these peaks, and it accounts very well for all of them except m/z 145:

m/z 186 = 100
157 = 28
158 = 23
131 = 22
187 = 13
139 = 12
128 = 11
185 = 11

The spectrum now has possible assignments for almost every peak. The tentative list is:

m/z 220:	$C_{15}H_{24}O$	nonyl phenol	
	$C_{15}H_{24}O$	branched alkyl phenol	Either or perhaps both
	$C_{14}H_{20}O_2$	alkyl benzoquinone	
m/z 208:	$C_{14}H_8O_2$	anthraquinone	Either or perhaps both
	$C_{15}H_{12}O_2$	methoxyphenanthrene	
m/z 186:	$C_{12}H_{10}O_2$	biphenol	

The search phase for this spectrum is now essentially complete in that it is probably not necessary to find more possible assignments, but the interpretation of the spectrum is incomplete as there are still choices to be made regarding the two undecided possibilities at m/z 220 and 208 and the possible assignment of the m/z 145 peak. Since the two possible identifications are in different reporting categories, it is worth some more effort to try to distinguish between them.

A choice between alternatives of this sort is most readily made in the confirmation or interpretation check phase of the analysis. Information from both IR and LRMS should be included.

The IR spectra may be able to provide an immediate basis for selection through specification of functional groups. However, the issue here would probably not be so easily settled, as phenols would be detected from the species at m/z 186, and from nonyl phenol as well. This might mask the presence of both species at either of the mass numbers in question. It would most likely be necessary to do reverse matching of the IR spectra to the unknown mix.

Reverse matching of the LRMS data with peak intensities taken into consideration may be able to decide the question. A match of this sort is shown in Table 13. The spectra of the tentatively identified components are scaled to have the base peak of the identified compound equal to the intensity of the corresponding peak in the unknown mixture. The two single identifications are first subtracted from the unknown, and the duplicates compared with the remainder.

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TABLE 13

SPECTRUM ACCOUNTING MATRIX

MIXTURE								
m/z	Intensity	Biphenol	Nonyl Phenol	Remainder	Branched Alkyl Phenol	Alkyl Benzoquinone	Anthra-Quinone	Methoxy-Phenanthrene
220	13		2	11	12	41		
209	22			22			15	17
208	100			100			100	100
205	50			50	50	50		
193	20			20				25
186	50	50		-				
180	38			38			74	
165	35			35				73
163	15		5	10		28		14
158	12	12		-				
157	15	14		-				
152	26			26			48	
151	13			13			24	
149	22		20	-		8		
145	12			12	4	11		
135	50		50	-				
131	11	11		11				
121	23		21	-				
107	25		23	-				
76	21			21			42	

For Page Numbering

It can readily be seen that the alkyl benzoquinone is probably not present, since it has two peaks, m/z 220 and m/z 163, that show substantial differences when compared to the unknown. In addition, the branched alkyl phenol is itself a good fit to the unknown, accounting very well for it. Hence, one chooses the branched alkyl phenol and disregards the alkyl benzoquinone possibility. The phenol also accounts for the otherwise unassigned m/z 145 peak.

The two species at m/z 208 both show patterns very similar to the unknown, but in both cases the fragments are about one half as intense as would be expected. Since none of the fragments of the two species coincide, but the base peaks do, the evidence suggests that both are present: if one divides the shown intensities by two and then adds both spectra, the fit to the remaining peaks of the unknown is quite satisfactory.

The "final" assignments (for this example) are shown in Figure 14 with an accounting of the spectrum. In the case of an actual sample, an IR evaluation of these identifications would be important at this point, as an important confirmation before the analysis report is written.

C. A Simple Mixture

This example is a demonstration of the analysis of an authentic but comparatively simple spectrum from LC fraction 3. Once again only a single spectrum, Figure 15, is considered. It is a scan from the lowest temperature part of the probe run, obtained just after the probe was

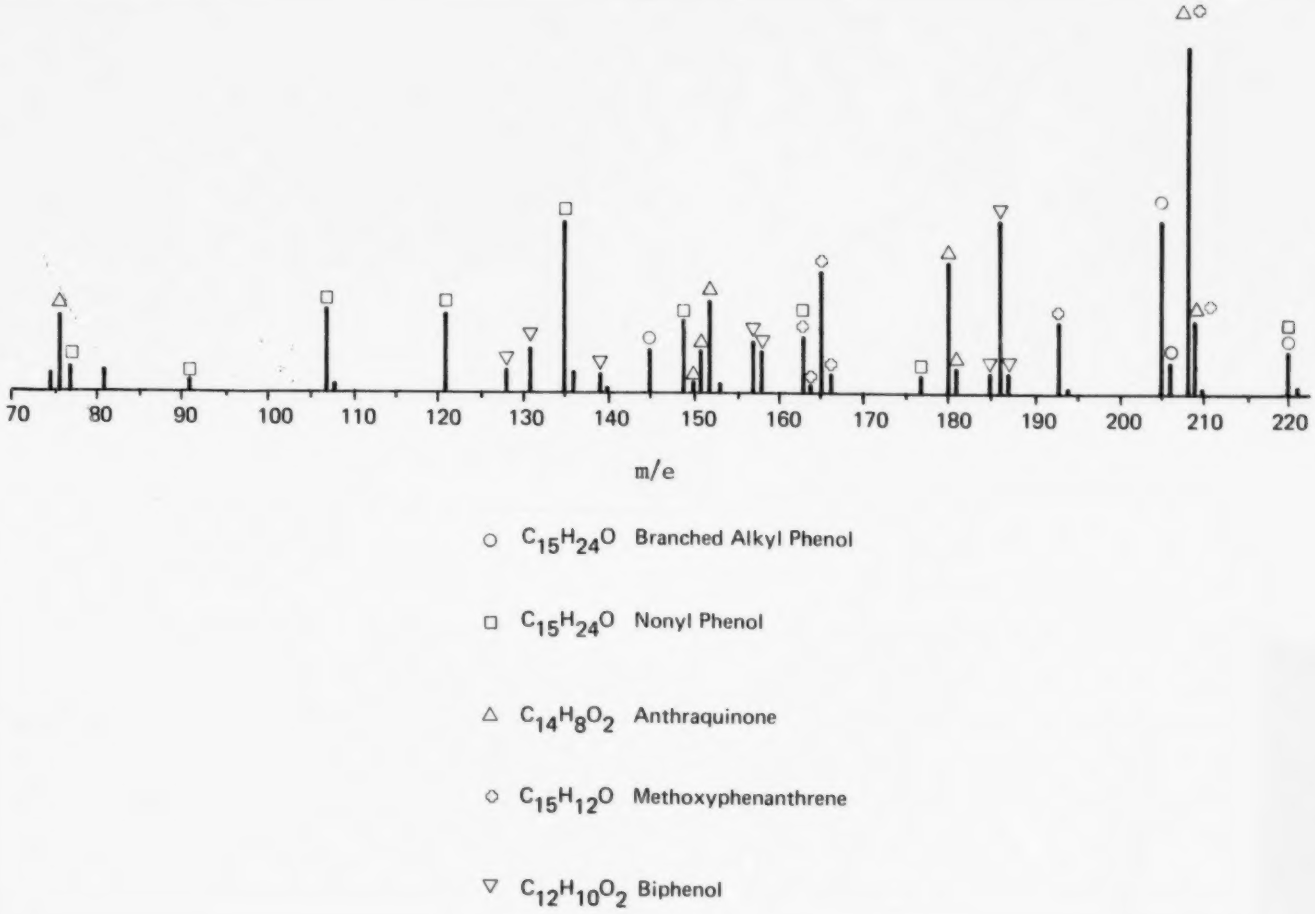

FIGURE 14 SPECTRUM ACCOUNTING

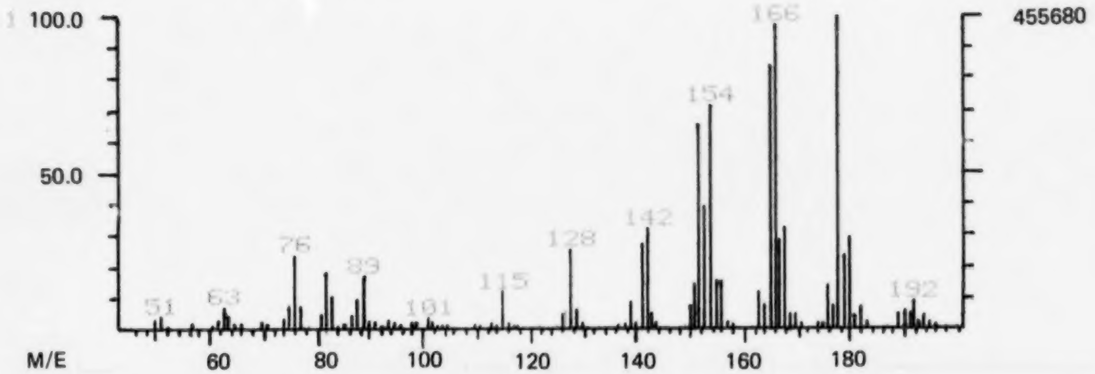

FIGURE 15 EXAMPLE #2: SPECTRUM OF A SIMPLE MIXTURE, LC FRACTION 3

inserted into the ion source. The ionization mode was 70 eV electron impact. No peaks were detectable at masses higher than m/z 204.

There are several initial observations to make: (1) this is an LC 3 fraction and therefore most likely to be predominantely of hydrocarbon composition. Sulfur and nitrogen are possible heteroelements, but if present will most likely be observable in LC 4 and LC 5 in greater concentration than is observed here. This example does not include spectra from later fractions, but it will be assumed for purposes of the demonstration that none of the peaks observable in this fraction will be seen at higher levels in later fractions.

(2) There are very few low m/z peaks. This indicates that there is very little fragmentation of the higher mass components, and suggests that they must be highly aromatic in character.

(3) All of the major peaks (except m/z 165), and most of the high mass minor peaks, have even number mass values. Since nitrogen is not a likely component of the compositions, the best first approximation is to assume that all of the high mass (>120) even number peaks are molecular ions.

The first tentative assumption is then that there are at least the following molecular weight components to identify:

128
142
152
154
156
166
168
178
180

Peaks which have high values for both intensity and mass will generally carry more information about the sample than those peaks which have low values for either mass or intensity. Consequently, in the analysis of multi-component spectra it is generally preferable to begin the analysis with those peaks that have the highest combination of mass and intensity. Accordingly, the first considerations are directed at m/z 178, through m/z 166, and so on.

Although the first assumption for this spectrum is that the major peaks are all molecular ions, a quick check for possible correlations between peaks, using Table 3, may be worthwhile. Listed in order of decreasing mass, the possibilities appear to be:

Primary Ion	Possible Correlations
178	192 - a homolog
166	152, 180, 194 - homologs
166	165 - fragment
154	168, 182 - homologs
154	153 - fragment
152	166, 180, 194 - homologs
152	151 - fragment
142	128, 156, 170 - homologs
142	141 - fragment
128	142, 156, 170 - homologs

It appears that there may be a few short series of alkylated components, starting with 128, 152 or 166 (since 166 is more intense than 152) one would not expect it to be a methylated 152, though such ratios are not conclusive), and 178. This is, however, not enough information for development of an identification.

The next step is to try matching spectra to the Eight Peak Index. In addition to the peaks listed as possible molecular ions, there are two possible single-chlorine isotope pairs that should be checked out. These are visible at m/z 166 and 168 and m/z 178 and 180. In both cases the ^{37}Cl peak is somewhat smaller than ideal, but the possibility of additive interference on the apparent ^{35}Cl peaks is sufficient to be able to account for the difference. (If chlorinated materials are present in the sample, they could very well appear in LC 3 if the hydrocarbon part of the molecule is sufficiently aromatic.)

The approach for using the Eight Peak Index is basically similar to that described in the previous example, in that the reference spectra are searched for possible identifications, and then the possible identifications are matched to the unknown for completeness of fit. The variant in this case is that the criteria for searching are slightly different.

Since the first test is to evaluate the mass pairs at m/z 166 and 186, and at 178 and 180 as possible single-chlorine-containing species, the Index is first searched for possible fits among those listings that have m/z 178 as the most intense ion, and which have one chlorine in the formula. (The fragment ion listing does not have composition indexing, but it is not difficult to scan as small a class as the 178 most-intense-ion one, for something as uncommon as a single chlorine in the composition.)

This search produces, for m/z 178, only 1-chloro-4-methylphthalazine as a possibility with a marginal fit. The search using a m/z 166 as the most intense ion yields no identifications that satisfactorily survive the reverse matching process. This lack of fit coupled with the lack of apparent chlorine isotopes anywhere else in the spectrum makes it quite unlikely that the peak pairs at 166 and 168, and at 178 and 180, represent chlorine isotopes.

The next step is to search the Index on the basis of peak intensities. None of the major peaks (>50%) show plausible losses from m/z 178, so for the second peak in the search, all of the peaks above 20% intensity of base ion will be tried. The only surviving fits are with 179 as the second peak:

Anthracene	$C_{14}H_{10}$
Phenanthrene	$C_{14}H_{10}$
Diphenylacetylene	$C_{14}H_{10}$

Moving down the spectrum to the next major peaks, trying them all as second peaks to m/z 166 yields only one possibility for m/z 166:

Fluorene	$C_{12}H_{10}$

Then m/z 154, with the major peaks as second intensity possibilities, yields:

Biphenyl	$C_{12}H_{10}$
Acenaphthalene	$C_{12}H_{10}$ (best fit)

When a similar search pattern is followed down to m/z 128, the following results are obtained:

m/z	Composition	Possible Assignments
178	$C_{14}H_{10}$	Anthracene/phenathrene/diphenylacetylene
166	$C_{12}H_{10}$	Fluorene
154	$C_{12}H_{10}$	Acenaphthene/biphenyl
152	$C_{12}H_8$	Acenaphthylene/biphenylene
142	$C_{11}H_{10}$ $C_{10}H_6NO_2$	Methyl naphthalene
128	$C_{10}H_8$	Naphthalene

These assignments also account for all of the major low mass fragment ions. The $C_{11}H_{10}$ identity for m/z 142 is chosen over the spectrally possible $C_{10}H_6NO_2$; the former is not only much more plausible in LC fraction 3, but is also much more consistent with the other assignments.

If the possible correlations observed earlier through the use of Table 3 are now reconsidered, the possible homologs appear to account for most of the minor peaks above m/z 120. There is an alkylated naphthalene series:

142
156
170

an alkylated fluorene series:

180 and possibly 194

and an alkyl anthracene/phenanthrene peak at m/z 192.

The materials tentatively identified, listed in order of apparent abundance, are thus:

Material	mw	Composition
3 ring PAH (e.g., phenanthrene)	178	$C_{14}H_{10}$
3 ring PAH (fluorene)	166	$C_{13}H_{10}$
3 ring PAH (acenaphthene)	154	$C_{12}H_{10}$
3 ring PAH (acenaphthylene)	152	$C_{12}H_8$
Alkyl 2 ring PAH (naphthalenes)	142-170	$C_{11}H_{10}$ - $C_{13}H_{14}$
Alkyl 3 ring PAH (or biphenyls)	168-182	$C_{14}H_{12}$ - $C_{15}H_{14}$
Alkyl 3 ring PAH (fluorenes)	180	$C_{14}H_{12}$
Alkyl 3 ring PAH (e.g., phenanthrenes)	192	$C_{14}H_{12}$

Smaller peaks that are not accounted for in this example are essentially unidentifiable without additional information. In the case of a complete analysis, the additional information is most likely to come from other scans at higher temperature, or perhaps from other ionization modes.

An experienced LRMS analyst would probably have been able to identify the components of this example by direct recognition of the spectra of the individual components, and would have needed only to check their spectra and confirm that the identifications adequately accounted for the observed peaks. Quick recognition of spectral components is a valuable skill that saves considerable time in the analysis, making interpretation of mixed species spectra a much more practical task than it could otherwise be.

D. A Complex Spectrum

This complex sample, whose spectrum (Figure 16) was chosen for illustrative purposes, has not been processed through the Level 1 LC separation scheme. However, the components present are representative of the materials observable in LC fraction 2, and perhaps also in Fraction 1. It is once again a single spectrum example, obtained with electron impact ionization of 70 eV and a probe temperature of about 80°C.

The first general observations that can be made regarding the spectrum are that:

1. The multitude of peaks of roughly comparable size suggests that it is a many component mixture in which no single component dominates;
2. There are roughly three envelopes of peaks:
   (a) a low mass, fragment ion group, m/z 43, 57, 71, 85, 97, 111, ...;
   (b) a middle mass group of 141, 155, 170;
   (c) a high mass group of 198, 212, 238, 252...

When these groups of peaks are correlated via Table 3, several further observations can be made:

3. The first peaks of the high mass group fall into the +2Z, column. This is the column of alkanes and alkyl naphthalenes. The ions of this series can actually be

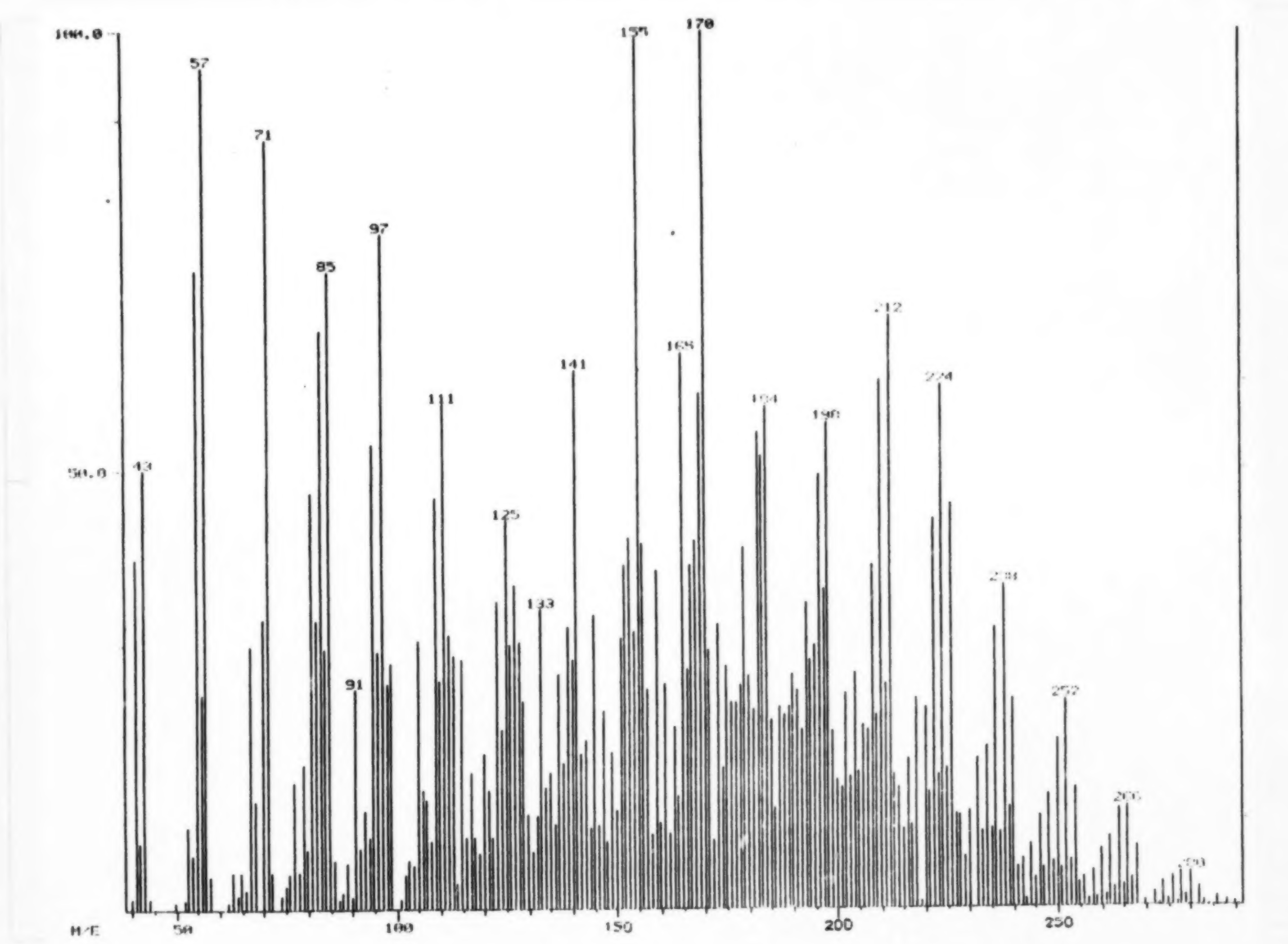

FIGURE 16 EXAMPLE #3: A COMPLEX SPECTRUM, PROBE TEMPERATURE 80°C

seen above the general clutter from m/z 156 through 268, although they are dominant only from m/z 170 through 212.

4. Starting at m/z 182 and continuing to m/z 280, there is a major series of peaks from the Z 0 column, characteristics of monoalkenes, cycloakanes, and cycloalkane naphthalanes.

5. From m/z 208 through 278 another series is visible. These peaks fall into the Z-2 column, and may be dienes, monoalkenes, cycloalkenes, cycloakene naphthalenes, or any of several other alkyl PAH series.

6. The middle group consists of m/z 141, 155 and 170. The m/z 170 peak falls in the Z+2 column while the m/z 131 and 155 peaks fall into the Z+1 column which is characteristic of fragments from Z+2 species. Since the peaks at 155 and 170 are largest in the spectrum, it does not seem possible that they could be alkanes. It is much more likely that they are alkyl naphthalenes.

7. The low m/z fragments are predominantly those of the C_nH_{2n+1} series at the low mass end, but with strong peaks from the C_nH_{2n-1} series that become the dominant fragment peaks from m/z 97 to perhaps 167. Both fragment series could be from either aliphatic or alkyl aromatic compounds.

8. There is also a somewhat smaller series of alkyl benzene fragment ions from the -7 column of the Z table: 91, 105, 133, 147,... (the fact that 119 is missing is probably due to the subtraction of the internal mass marker compound from this spectrum).

9. A minor series of peaks from the Z-9 column is observable: m/z 145, 159, 173, and perhaps higher. This column position suggests alkylated indanes. A check of the Eight Peak Index for spectra of several molecular weight values for that class which appear to be present in the spectrum (188, 202, 216), uncovers the additional possibility that the group could also come from alkyl tetrahydronaphthalenes.
10. There is a major peak at m/z 165 that has not been accounted for in any of the classes discussed to this point. It could be a fragment ion from fluorene and methyl fluorene. This interpretation is consistent with the size of the 165 fragment and 166 and 180 molecular ions.

All that has been observed so far suggests that this is a spectrum of a hydrocarbon mixture. The basic decision that needs to be made before the interpretation can proceed much further, is whether the high mass peaks are from aliphatic species, or from alkylated aromatic species. This choice can be made on the basis of comparison of the intensities of the low mass alkyl ions with the intensities of the high mass molecular ions. Alkylated aromatic species show comparatively few low mass fragment ions, particularly if the aromatic unit is as large as or larger than naphthalene. Aliphatic species are just the opposite in that they show very intense low mass fragment ions, and very small molecular ions. In this example, the fragment ions are only slightly more intense than the molecular ions as they appear in the spectrum. Also, in this spectrum taken at 70 eV ionizing voltage, the low molecular weight fragment ions of aliphatic compounds would be expected to be many times as large as the molecular ions. In addition, the fragments would, for the most part, be common to all of the different molecular weights. Thus additive interference will, in an aliphatic mixture, build the fragment ions to values very much more intense relative to the parent ions than would be expected from a single species. This clearly has not happended to any significant degree in this spectrum. (It is not possible to conclusively eliminate the possibility of aliphatic species, due to the very small molecular ions that would be present from the observed fragments. However, considering the many possibilities of contribution to the low mass fragments from the assigned classes with parent ions in the range of m/z 170 to 280, it would appear that all of the low mass ions could readily be accounted for without postulating the presence of aliphatic compounds.)

On the basis of the information obtainable from this spectrum, the identification of this fraction must be limited to chemical class and molecular weight range. Without additional information that could perhaps be supplied by thermal fractionation, soft ionization techniques, or the IR data and TCO GC plots, nothing more specific can reliably be determined. There are simply too many peaks and too many components present for reference spectrum matching, or for any other than general MS interpretation features to be useful. The degree of identification achieved is adequate for the purpose of the Level 1 program, since the chemical classes have been determined for most of the sample, and the comparative intensity level of those classes have been estimated as well.

The summary for this example is as follows, in order of decreasing abundance:

Subcategory and Specific Compounds		mw	Composition
Alkyl napthalenes		142-282	$C_{11}H_{10}$ - $C_{21}H_{30}$
Alkyl Benzenes		>134	> $C_{10}H_{14}$
Possible PAH:	3 ring	152	$C_{12}H_8$
	3 ring	166	$C_{13}H_{10}$
	Methyl 3 ring	180	$C_{14}H_{12}$

E. A Confusing Mixture

The fourth example is a multispectral one. The sample spectra are shown in Figures 17 to 20, covering a temperature range of 50° C to 80°C. The spectra from the preceeding LC fraction (LC2) at a comparable temperature are shown in Figures 21 and 22. Two spectra from the subsequent LC 4 fraction, also obtained at a comparable temperature, are shown in Figures 23 and 24. All spectra were obtained at 70 eV shortly after introduction of the sample via the direct insertion probe. The fraction is LC 3, from cigarette tar.

This set of spectra is confusing for two principle reasons: (1) the mass values are not typical for aromatic fused hydrocarbons. They may, with approximately equal likelihood, be oxygen- or nitrogen-containing species, judged only on spectral appearance; and (2) the intensity ratios among all the peaks appears to change with each scan. This latter fact makes the determination of peak association a

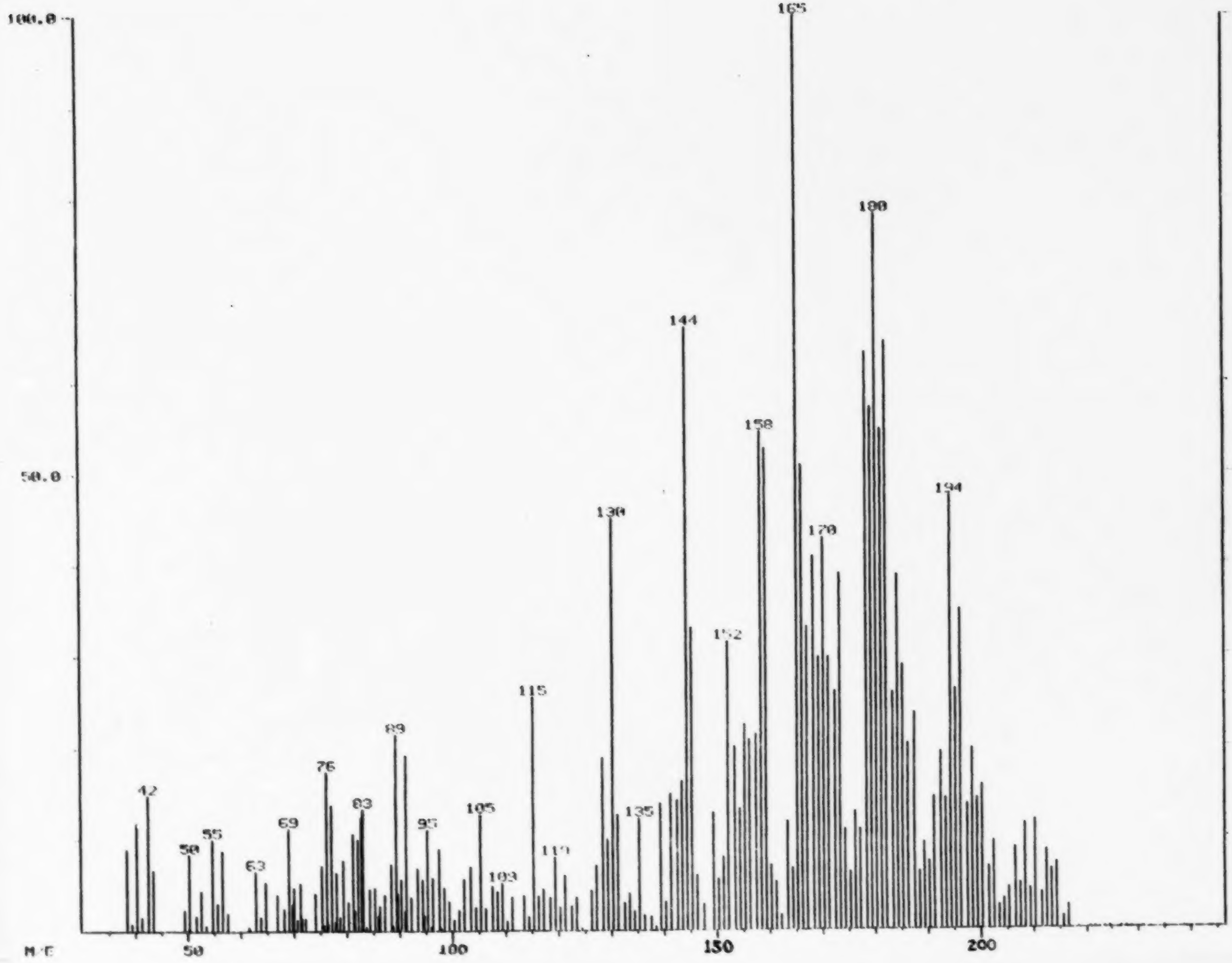

FIGURE 17 EXAMPLE #4: CIGARETTE TAR, LC FRACTION 3, PROBE TEMPERATURE 50°C

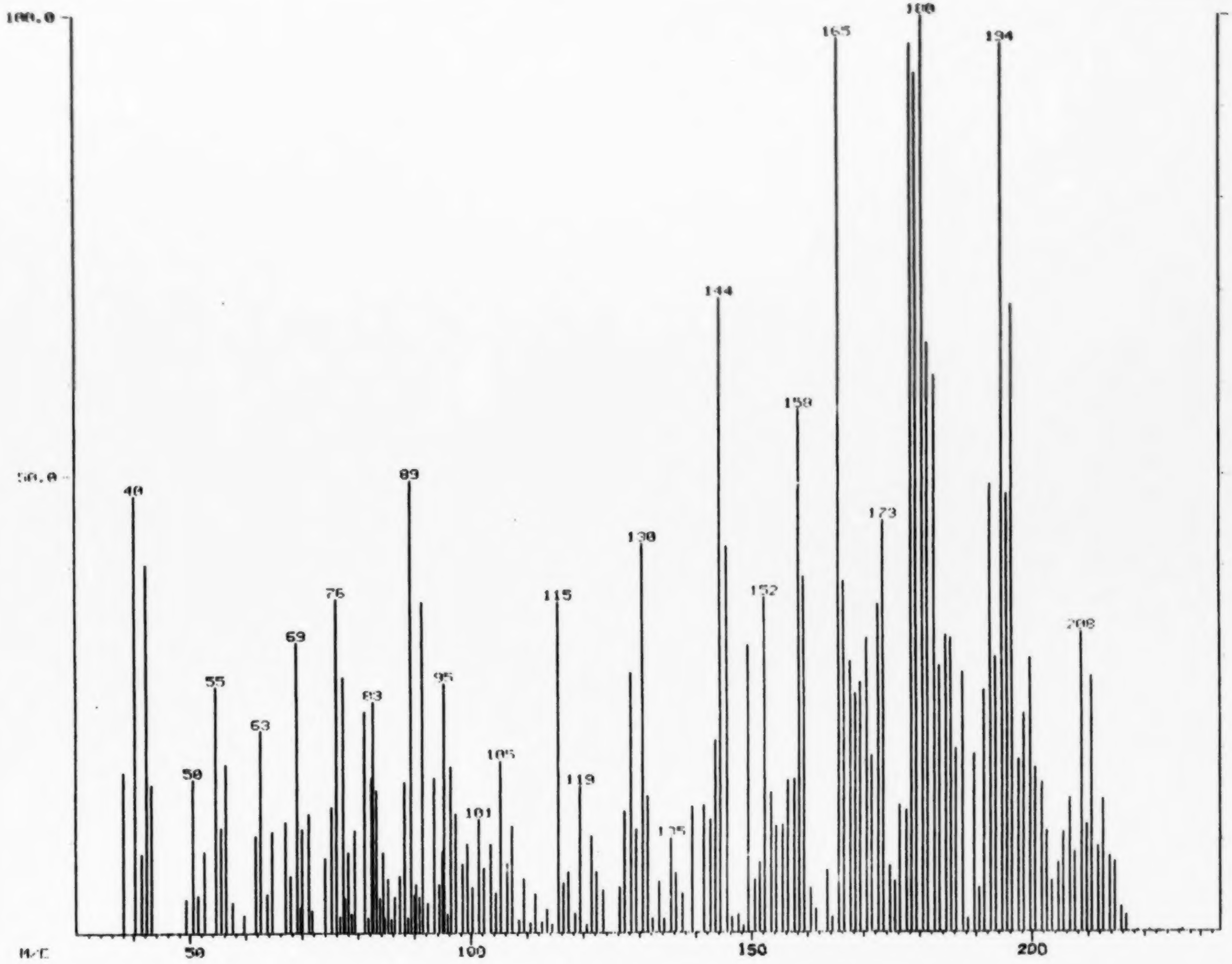

FIGURE 18 EXAMPLE #4: CIGARETTE TAR, LC FRACTION 3, PROBE TEMPERATURE 60°C

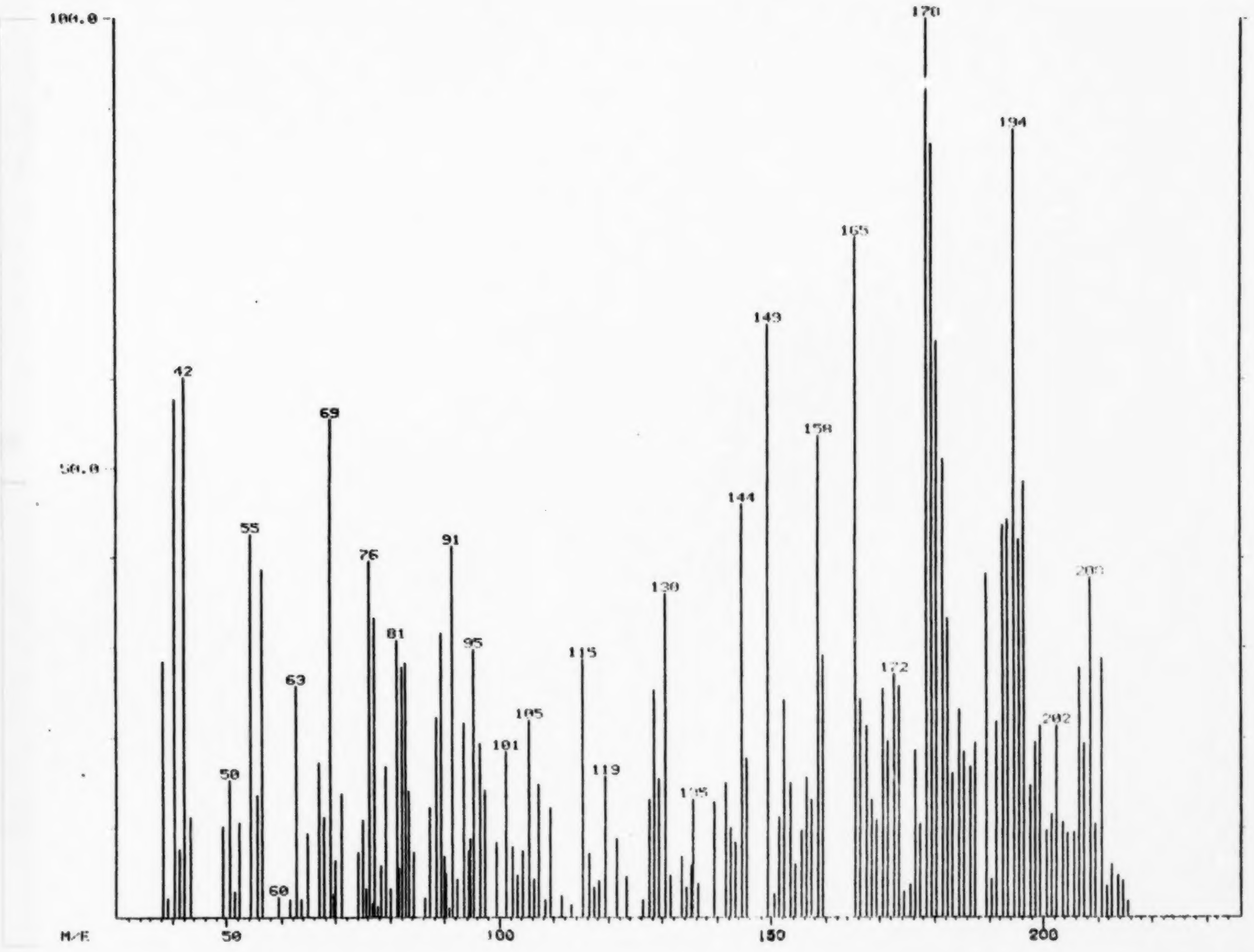

FIGURE 19 EXAMPLE #4: CIGARETTE TAR, LC FRACTION 3, PROBE TEMPERATURE 70°C

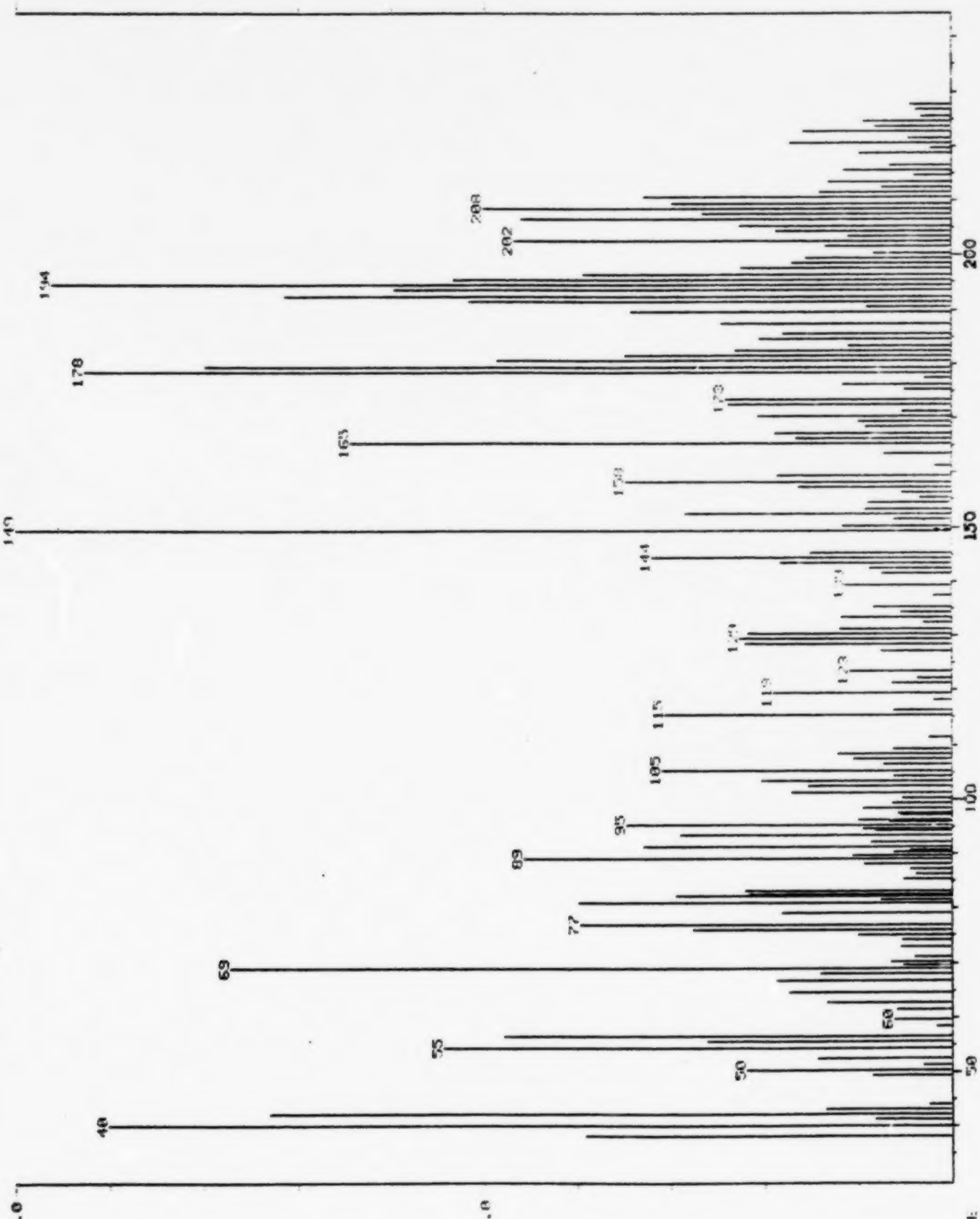

FIGURE 20 EXAMPLE #4: CIGARETTE TAR, LC FRACTION 3, PROBE TEMPERATURE 80°C

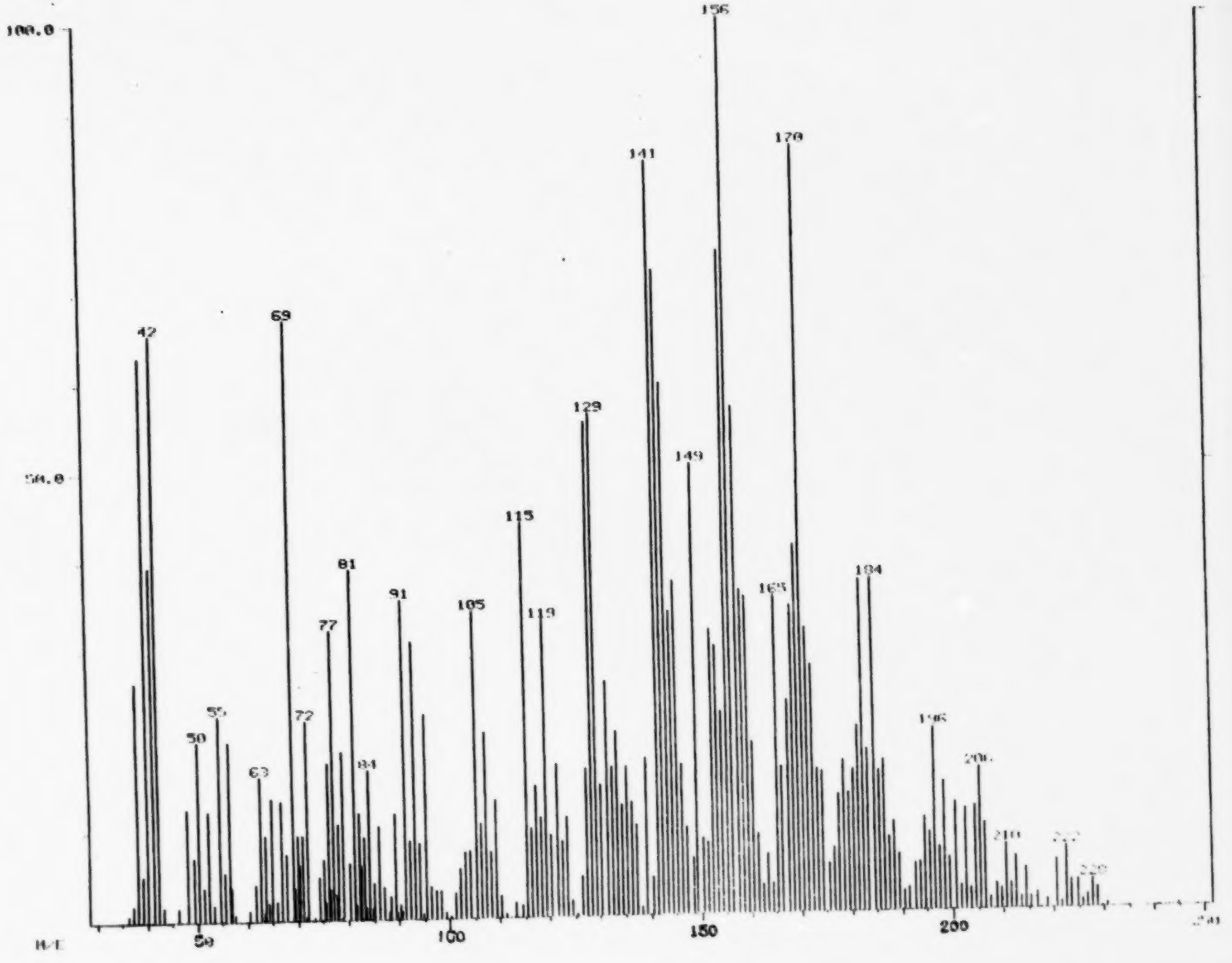

FIGURE 21 CIGARETTE TAR, LC FRACTION 2, PROBE TEMPERATURE 60°C

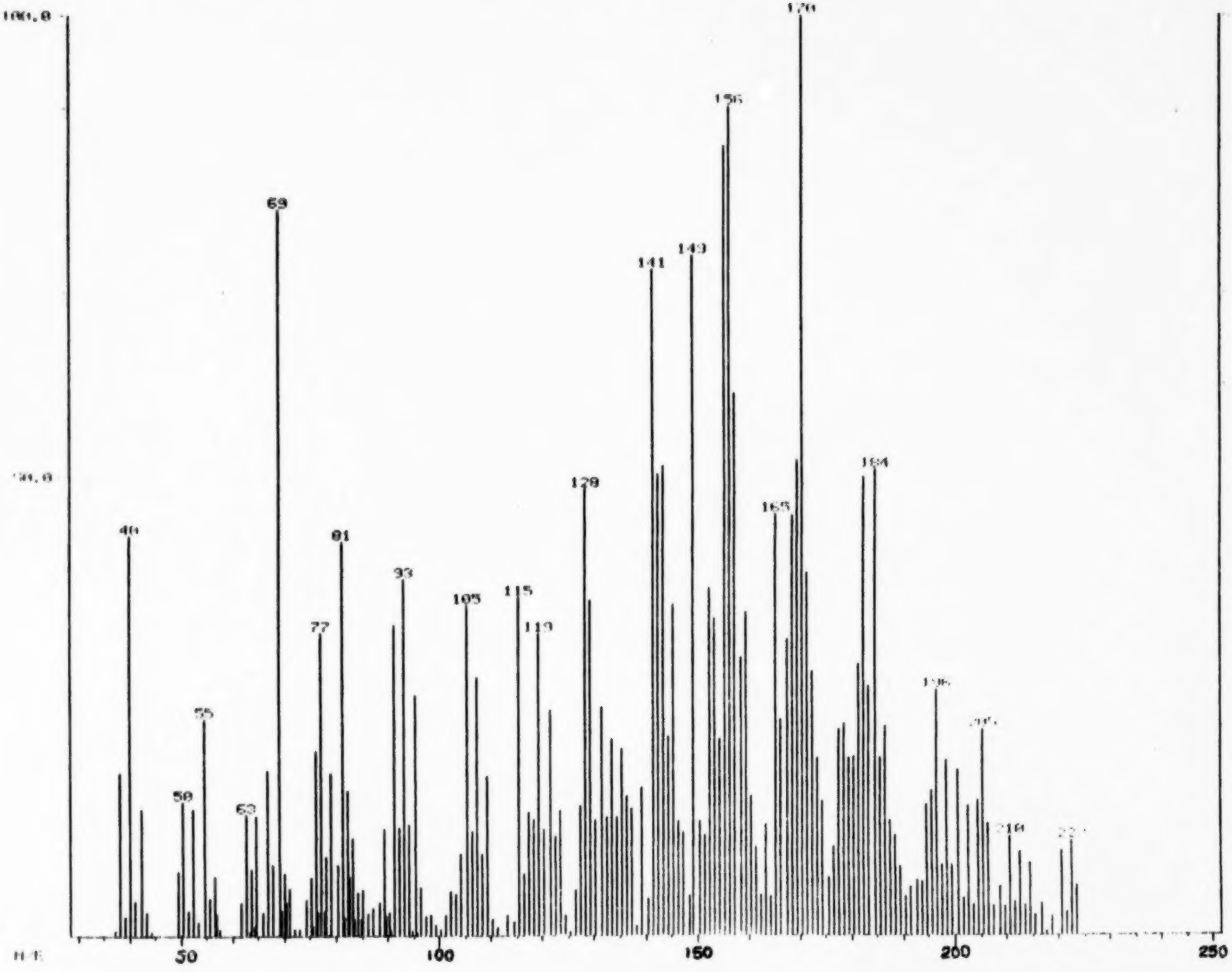

FIGURE 22 CIGARETTE TAR, LC FRACTION 2, PROBE TEMPERATURE 70°C

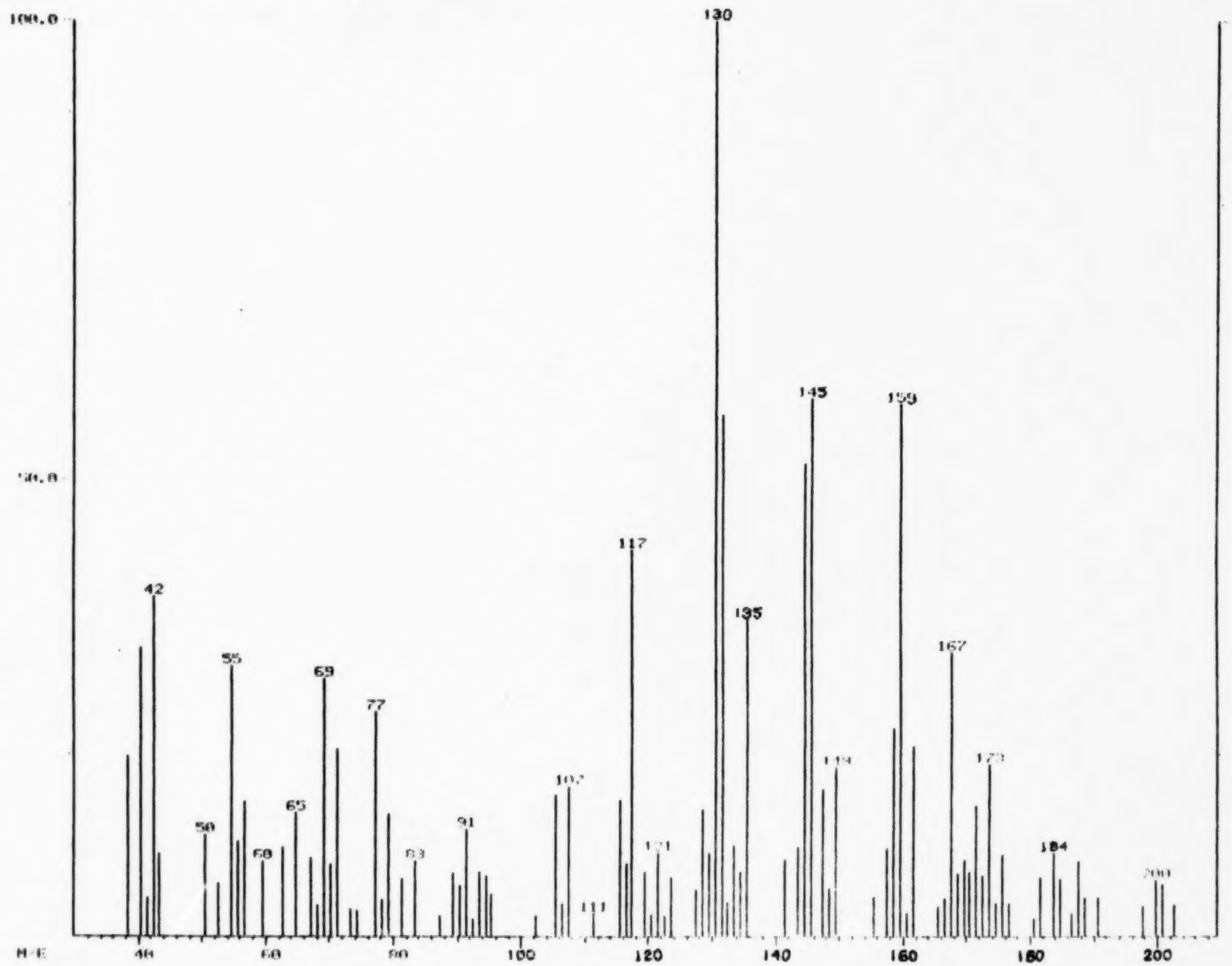

FIGURE 23 CIGARETTE TAR, LC FRACTION 4, PROBE TEMPERATURE 60°C

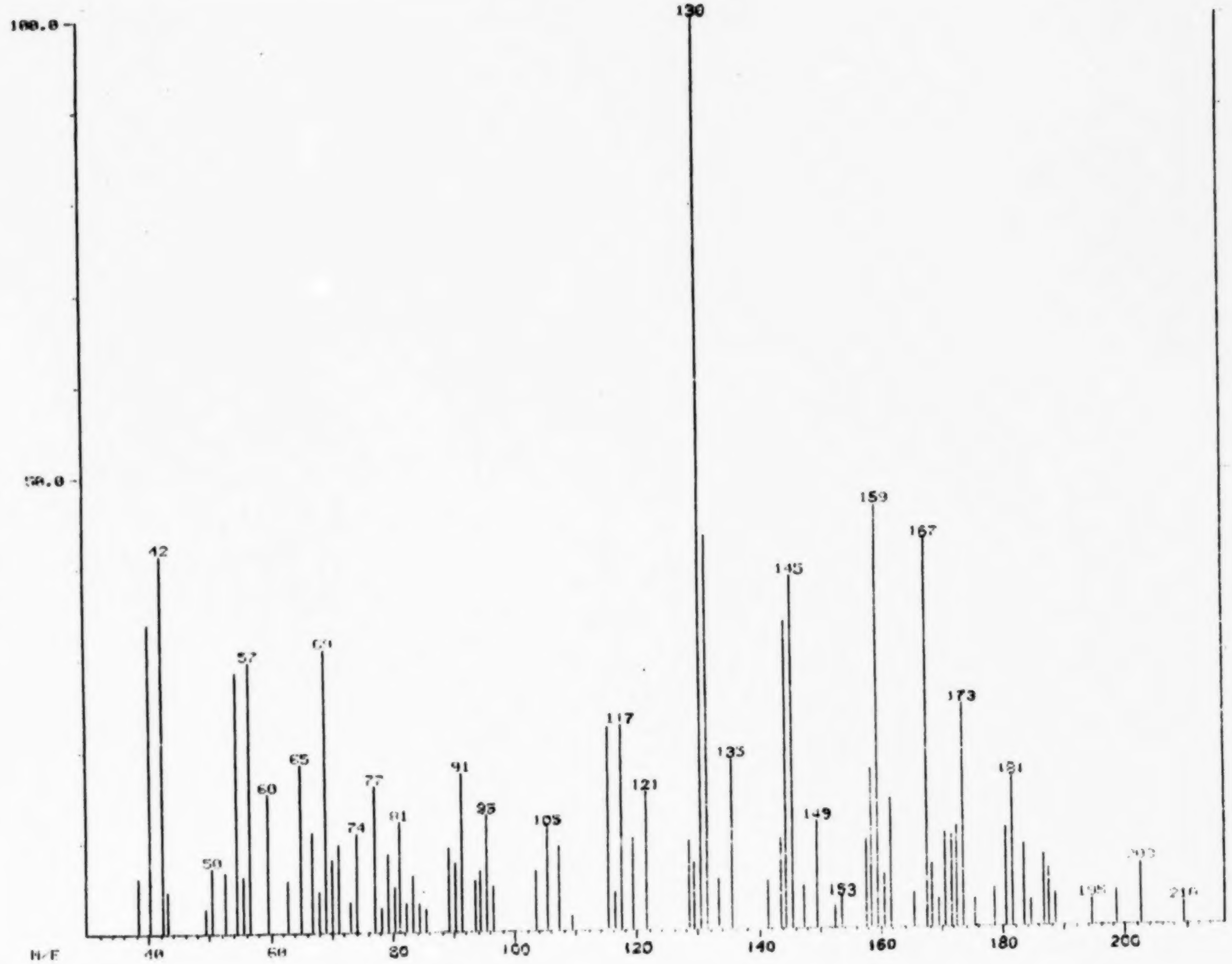

FIGURE 24 CIGARETTE TAR, LC FRACTION 4, PROBE TEMPERATURE 70°C

difficult matter.

The fact that the fraction is LC 3 cannot be completely relied upon to exclude the possibility of nitrogen- or oxygen-containing compositions. Cigarette smoke condensate is known to contain oxygen and nitrogen polycyclics in concentrations as great as or greater than those of the PAH series.[37] An important clue in this regard can be obtained by comparing equivalent spectra from LC 4. If oxygen or nitrogen polycylics are present in LC 3, they should also be present in LC 4 and probably in greater abundance. When this comparison is made in Figures 23 and 24, some species which apparently contain nitrogen are readily observable at m/z 117 (indole?), m/z 131, 145, 159, 173 (alkyl indoles?), and at m/z 167 (carbazole?) in the spectra of LC 4. But the only prominent peaks from LC 3 that are also present in LC 4 are m/z 130, 144, 145, and 159. Therefore, a tentative conclusion can be taken that it is only these few peaks in the spectra of LC 3 that correspond to nitrogen-containing compounds; most of the peaks in the spectra being analyzed are hydrocarbons.

In most cases IR might be expected to resolve the question of heteroelements. In this case that was not possible; this was a dilute fraction which, at higher probe temperatures, was dominated by a phthalate. The IR spectrum was that of the phthalate. The components being studied here were simply too small in the phthalate mixture to be assignable by IR.

As was stated in the analysis of the second example, the peaks most effective in the analysis of mixed species spectra are those with the highest combination of mass and intensity. In this example those peaks exhibit relationships that appear to be continually changing, and so are difficult to assign. An alternative approach offers more promise here. When a series of ions can be observed to what appears to be a lowest molecular weight member, there can be advantage to pursuing the identification of that lowest component. Since it is the lowest weight, it will intrinsically have the fewest possible assignments for composition, and probably for structure as well. When the base ion of the series is in an uncluttered region of the spectrum, spectral matching may be useable as an identification mode.

In this example, m/z 130 appears to be such an ion. It is apparently the lowest member of a series that includes m/z 144, 158, and possibly 172, 186 and 200. The members above m/z 130 increase on a comparative basis with increasing probe temperature (each succeeding scan represents a slightly higher temperature). When the increase with increasing temperature is observed first for m/z 144 and then for m/z 158, it becomes apparent that m/z 145 and 159 are also involved, and then that m/z 172 and 173 may be as well. This seems to confirm the idea that m/z 130 is the base unit of a series of alkylated compounds, but it also suggests that the molecular ions may be the odd numbers: m/z 145, 159, and 173.

An attempt is now made to correlate m/z 130 with possible lower fragment ions, particularly with the apparently plausible m/z 115. This fails for two reasons: first, m/z 115 increases with increasing temperature, while m/z 130 does not. In fact, virtually all of the lower mass ions increase similarly. Second, the Eight Peak Index does not have any m/z 130, 115 entries that can fit the spectrum. The methyl indenes are the only entry that match the 130, 115 criterion; however, they have much higher peaks at 128 and 129 than are observed in the spectra. The odd-molecular-weight clue turns out to be the answer; further work with the Index establishes that there is a good fit for the series as alkyl indoles. The assignments for each peak in the series are as follows:

m/z	Assignments
130	1/2 methyl indole fragment, 1/2 C_2 alkyl indole fragment
131	methyl indole molecular ion
144	1/2 C_2 alkyl indole fragment, 1/2 C_3 alkyl indole fragment
145	C_2 alkyl indole molecular ion
158	1/2 C_3 alkyl indole fragment, 1/2 C_4 alkyl indole fragment
159	C_3 alkyl indole molecular ion
172	1/2 C_4 alkyl indole fragment, 1/2 C_5 alkyl indole fragment
173	C_4 alkyl indole molecular ion
etc.	

The series may extend as far as m/z 201, but the spectrum in the higher mass area is too crowded with peaks of other species for accurate evaluation.

The alkyl indole assignments account for the major peaks between m/z 120 and 160, and a few of those above 160. But the majority of the high mass peaks remain confusing. The major peaks are not definitive on the basis of simple mass value, and the ratios between them change with every scan, making association of potential molecular and fragment ions very difficult. When the ratios between peaks change in this manner, it indicates either that they arise from different species, or that at least one of the two peaks being ratioed has contributions from more than one species.

The latter appears to apply to the two major peaks of the first scan, Figure 17; m/z 165 is first 20% larger than m/z 180, then about the same size (Figure 18), then 15% larger (Figure 19), and finally about 20% larger again (Figure 20). The Eight Peak Index lists only two hydrocarbon species that have m/z 165 as the base peak; namely, methyl acenaphthylene and methyl fluorene. A third species with a large but not a base m/z 165 peak is fluorene. m/z 166 in the first scan is approximately 50% of the 165 peak. It then declines steadily with each scan until the fourth scan it is less than 25% of m/z 165. The 180 peak attains a maximum (in comparison with the other peaks) on the second scan, and then both it and m/z 165 fall off rapidly in intensity with succeeding scans. All of this behavior can be accounted for if one assigns identifications of methyl fluorene to m/z 180, and either methyl acenaphthylene or fluorene to m/z 166. These two compounds have spectra that are very similar, and cannot be distinguished in a mixture of this sort. However both are PAH materials with a composition of $C_{13}H_{10}$, and both have 3 rings. This degree of identification is ample for the needs of the Level 1 program.

One might seek to correlate m/z 152 with m/z 180. The ratios between the two are reasonably constant over the first three scans (41%, 37%, 39%) changing only in the fourth. The ratio between these two peaks is characteristic of fluorenone; but if a ketone is to appear in LC fraction 3, it should also appear in the later fractions. This is definitely not occurring here, so fluorenone can be excluded as a possible identification. It is more likely that m/z 152 is due to acenaphthylene (or biphenylene), $C_{12}H_8$. This assignment is consistent with the presence of the $C_{13}H_{10}$ species at m/z 166. It is also consistent with the appearance in the earlier LC 2 fraction (Figures 21 and 22), of m/z 152 and the m/z 165, 166 pair, without the major 180 peak.

The peaks that are next most intense overall are m/z 178, 179, and 194. As LC 3 is the fraction in which the PAH materials are most likely to appear if they are present in the sample, and this is a sample which is known to contain PAH materials, it is reasonable to first evaluate m/z 178 as an anthracene/phenanthrene peak. This assignment is consistent with the reference spectra; there are obviously interfering species at m/z 179, but the peaks at 176 and 177 appear to be clear in the last two scans where m/z 178 is most abundant, and a good match to the reference spectra. This identification is reinforced by the appearance in the last two scans (Figures 19 and 20) of the related species peak of pyrene/fluoranthene at m/z 202. These identifications in turn suggest that the peaks at m/z 192 and 191 are most likely due to methyl anthracene/phenanthrene, and that those at m/z 206 and 205 are assignable to C_2 alkyl anthracene/phenanthrene.

The few remaining major intensity peaks are at m/z 167, 169, 170, 171, 179, 182, 181, 184, 185, 194, 195, 196, 208, and 210. Except for m/z 185 and 171, all of these peaks appear to belong to no more than three series (Table 3). The series are the alkyl naphthalene series, m/z 169, 170, 184 (there appear to be other members present at lower intensity levels); another molecular ion and fragment ion series of m/z 167, 168, 181, 182, 195, 196, and 210; and the alkyl acenaphthylene/fluorene series already identified at lower masses, here represented by m/z 179, 194, and 208.

The alkyl naphthalenes were an abundant component of the previous fraction (Figures 21 and 22), and here in this fraction show matches to the reference spectra that are consistent with the interpretation. It is not reasonable to expect to be able to obtain identifications on the basis of spectral match alone when the peaks being matched are surrounded by other peaks of similar or greater intensity. The most that can be established is whether the assignment is consistent with the reference standard; such consistency is necessary but not sufficient for identification. Identification in these instances requires that there be other supporting information. This may be obtained from several sources, but will most often be the identification of the compound as a major component from other spectra that are part of the same continuum as that being analyzed. The most common of these are spectra taken at different temperatures, from adjacent fractions, or from alternate ionization techniques.

The alkyl PAH series assignment is similarly plausible on the basis of the m/z 178 and 202 PAH assignments, the LC fraction, the reference spectra, and the source origin consistency.

The molecular and fragment ion series of m/z 167, 168, 181, 182, 195, 196 and 210 remains to be assigned. These are from the Z=0 and -1 series (Table 3) and could in this fraction be alkyl biphenyls or acenanphthenes. Alkyl dibenzofurans or alkyl benzophenones are also within the range of possibility, but only if present as a pre-elution of a later LC fraction. The ions of this series are consistent with the reference spectra for alkyl biphenyls.

This leaves m/z 171 and 185 unassigned among the larger peaks, and many smaller peaks as well. These peaks must remain unclassifiable in spectra that show no better component separation than is visible here.

In the later scans (Figures 19 and 20) the characteristic m/z 149 phthalate ion is becoming dominant.

The complete set of assignments for these for sample spectra is:

Compounds Identified	mw	Composition
Alkyl 3 ring PAH, C_1, C_2	180-194	$C_{14}H_{12}$ - $C_{15}H_{14}$
3 ring PAH (e.g., Anthracene/ Phenanthrene)	178	$C_{14}H_{10}$
Phthalate, not specifically identifiable		
Methyl 3 ring PAH	192	$C_{15}H_{12}$
Alkyl indoles	145-159	$C_{10}H_{11}N$ - $C_{11}H_{13}N$
Alkyl biphenyls	182-196	$C_{14}H_{14}$ - $C_{16}H_{16}$

Compounds Identified	mw	Composition
Alkyl indoles	131, 173-201	C_9H_9N, $C_{12}H_{15}N$ - $C_{14}H_{19}N$
Alkyl 3 ring PAH	166, 208	$C_{13}H_{10}$, $C_{16}H_{16}$
Alkyl biphenyls	168, 210	$C_{13}H_{12}$, $C_{16}H_{18}$
Alkyl naphthalenes	142-198	$C_{11}H_{12}$ - $C_{15}H_{20}$
4 ring PAH (e.g., Pyrene/ Fluoranthene)	202	$C_{16}H_{10}$
3 ring PAH (Acenaphthylene)	152	$C_{12}H_8$

Other

Many unidentifiable minor peaks, m/z 160 to 200.

CHAPTER V

THE LEVEL 1 LRMS REPORT

A. Introduction to the Report

A standard form is used for reporting the results of the LRMS analysis of each LC fraction analyzed.[1] This form is shown in Figure 25. The analyst is called upon to specify the chemical class of all of the materials observed in the fraction, with estimates of quantity present given on a relative basis of 100, 10, and 1. The materials observed are reported qualitatively by category, subcategory and/or specific compounds, and "other." For the Level 1 LRMS report, chemical class and category are synonymous.

The Level 1 reporting categories are also standardized for uniformity in reporting and interpretation. The complete list of standard categories along with a representative sampling of subcategories, is shown in Table 14. The choices of categories have been adapted from the MEG 39 list with minor modifications for greater compatibility with mass spectral data. The category list is fairly comprehensive in range and will be found adequate to cover the chemistry in most samples. The analyst is, however, not restricted to the specified listing; additions can be made as necessary. Addition should not be made arbitrarily, but rather only if the material identified cannot be correctly classified into one or more of the categories of Table 14.

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LRMS REPORT

SAMPLE: ____________________

Major Categories

Intensity	Category	MW Range

Sub-Categories, Specific Compounds

Intensity	Category	m/e	Composition

Other

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FIGURE 25 LEVEL 1 LRMS REPORT FORM

For Page Numbering

TABLE 14

CATEGORIES FOR REPORTING LRMS DATA

Category (Subcategory)	Most probable LC fraction*	Category (Subcategory)	Most probable LC fraction*
Aliphatic hydrocarbons	1	Phenols	6
(Alkanes)	1	(Alkyl, etc.)	6
(Alkenes)	1	(Halogenated phenols)	6
(Alkynes)	1	(Nitrophenols)	6
Halogenated aliphatics	1,2	Esters	6
(Saturated)	1,2	(Phthalates)	6
(Unsaturated)	1,2	Ketones	6
		Amines	6
Aromatic hydrocarbons	2,3	(Primary, secondary, tertiary)	6
(Benzenes)	2,3	(Hydrazines, azo compounds)	6
Halogenated aromatic hydrocarbons	2,3	(Nitrosoamines)	6
Nitro aromatic hydrocarbons	4,5		
		Heterocyclic nitrogen compounds	
Fused alternate, nonalternate hydrocarbons	2,3	(Indoles, carbazoles)	4
MW < 216 (methyl pyrene)	2,3	(Quinolines, acridines)	6
MW > 216	2,3		
		Alkyl sulfur compounds	6
Ethers	4	(Mercaptans)	6
(Halogenated ethers)	4	(Sulfides, disulfides)	6
Epoxides	4	Heterocyclic sulfur compounds	
		(Benzothiophenes)	4
Aldehydes	4		
Heterocyclic oxygen compounds	3,4	Sulfonic acids, sulfoxides	7
Nitriles	4	Amides	6
(Aliphatic)	4		
(Aromatic)	4		
		Carboxylic acids	6,7
Alcohols	6		
(Primary, secondary, tertiary)	6	Silicones	2,3,4
(Glycols)	6		
		Phosphates	5,6,7

*Possible assignments. Fractions 4-5, 5-6, 6-7 generally overlap to a considerable extent. Also, additional components of a particular molecule may cause it to elute in an LC fraction other than that expected. For example, a short-chain ester would probably elute in LC fraction 5 or 6 whereas a long-chain ester would elute in Fractions 3 or 4.

The subcategories shown in Table 14 are representative only. There are actually many more subcategories possible than are listed in the table. The analyst is, at this level, free to describe the best specificity that can be achieved in identification of the sample components.

A category for Other is also provided in the LRMS report. In this area the analyst may specify those components of the spectrum, if any, that could not be identified. Other remarks pertinent to the analysis of the sample can also be placed here, if such comment seem advisable.

The report for each LC fraction needs to be internally consistent; all categories reported, including unclassified, should normally have expanded information given in the subcategory, specific compound, or "other" level. Conversely, all entries at those levels need also to be reported at the category level.

B. Intensity Reporting

The intensity levels to be used for reporting are:

Intensity	Significance
100	A major component in the LC fraction
10	Present in the LC fraction
1	A trace component of the LC fraction

These intensity levels are to be used in reporting at all levels of the LRMS Level 1 report, for category, subcategory and specific compounds, and Other.

Specific rules are not given for the assignment of these intensity levels. The individual analyst is expected to assess relative abundance on the basis of the performance of the instrument used, and on the characteristics of mass spectra in general. For many components a straightforward observation of component peak heights will provide an accurate enough basis for estimating intensity values. Allowance must, however, be made for the fragmentation characteristics of the species or class being evaluated. The standard for comparison can well be those species that show negligible fragmentation, such as the highly aromatic species that give essentially only one major peak in the spectrum. Then for those materials that show only a few major ion peaks, an estimate of the combined total of those peaks can be used as representative of that species or class. Materials that undergo extensive fragmentation into many ions, such as the aliphatic hydrocarbons, alcohols and others, will need a correction factored into their peak height values in order to make them comparable to the less fragmented species or classes.

If the LRMS system being used for Level 1 analysis is equipped with a data system that is capable of adding spectra, the task of evaluating total relative LC fraction component intensities can be considerably simplified. All of the spectra taken during the probe run can be added into one composite spectrum, which will represent an integration of the probe run. The intensities observed there then need only to be compared to the batch inlet data, if any, for total fraction evaluation.

No general rule can be given for relating the intensity values observed from the batch inlet to those observed from direct probe introduced samples, as there is too much variability between instruments in this regard. The individual analyst will need to do a few simple experiments for the specific LRMS being used. A complicating element in such an evaluation will come from those species that show variable degrees of sample loss during solvent drydown for the probe, depending on drydown rate and total drying time.

C. Molecular Weight Range Reporting

A molecular weight range is to be specified for each category, subcategory or specific compound, and Other reported. The molecular weight range refers to the chemical molecular weight range of the materials being reported, and not the range of parent and fragment ions observed for the class. At the category level of reporting, the entire molecular weight range observed for each category is reported.

When a category encompasses more than one intensity value over its entire molecular weight range, then the two (or three) ranges and intensity values are to be listed separately in the subcategory section of the report. If a lesser intensity value molecular weight range is contained wholly within a greater intensity value molecular weight range, it is not necessary to report the lesser value separately. For example, the following case:

Intensity	Subcategory	MW Range
100	Alkanes	226-338
10	Alkanes	240-324
1	Alkanes	254-296

would require only the first entry to be listed, as the two of lesser intensity are covered by and included in the listing of the greater intensity molecular weight range. However data which called for a listing of

Intensity	Subcategory	MW Range
100	Alkanes	184-268
10	Alkanes	184-310
1	Alkanes	310-366

would all require separate listings. The mw range overlap reported for the 10 intensity level entry would be needed only if subcategories or specific compounds different than those of the 100 intensity level were to be reported within the overlap range. If this were not the case, then

10	Alkanes	268-310

would be the suitable entry for that intensity level.

Molecular weight ranges, or apparent molecular weight ranges should be listed for unclassifiable materials. In general, as much information as possible should be listed for unclassifiable materials, as it may become interpretable later.

D. Category Reporting

The category level of the LRMS report for each LC fraction analyzed is of major importance, as it is this level that is combined with the TCO and GRAV data to produce the integrated Level 1 Report. The standard categories for reporting are shown in Table 14. The categories shown in the table should be used in the form given in the table. It is important that the fraction be reported comprehensively at the category level; that is, all of the material observed in the mass spectra of the entire LC fraction (batch and direct probe data) should be accounted for. If a portion of the spectra remains unidentifiable after best effort of analysis, then it should be evaluated for relative proportion to the classified components, and reported as "unclassified" with an accompanying intensity estimate and the apparent molecular weight range. An unclassified components may be omitted from the category section of the report if the intensity value is only 1, unless it appears to be of unusual interest.

The entries into the category section of the report are to be listed in order of abundance both within an intensity level, and overall. If a second ordering is needed it should be based on increasing molecular weight.

Problems in assigning category may be encountered when an identified species has two or more functional groups that will qualify it for more than one category, or when a portion of the spectrum may be identifiable only to the extent of either/or, involving more than one category class of functional group.

The general rule for these situations may be derived from the understanding that Level 1 is a survey scheme that is intended to flag effluents for further investigation. Thus false positives are to be preferred over false negatives when a choice has to be made between the two. It then follows that a species known to be multifunctional should be reported in all appropriate categories, except where one implies another. A methoxy-hydroxy naphthalene would be reported both as an ether and as a phenol, but not as a PAH since the aromatic function is implied in the phenol category. The intensity value should be reported as the single function value, divided by the number of functions (categories) being reported. Thus the component above, with an intensity value of 100, would be reported as 50 ethers, 50 phenols.

Cases of specific multifunctionality will be unambiguously reported if the internal consistency rule is followed; if one has enough specificity in the identification to establish the multifunctionality of the species, then one will also have enough information to list the functions accurately in the specific compounds section. A later investigator who may wish to pursue the phenol or ether category in the above case will then find the relevant detail listed under specific compounds that may be needed for further study.

The case of the not quite identifiable spectrum is less easily defined. When a spectrum is first identifiable only as an either/or functionality, a choice between the two or more possibilities can usually be made by comparing the spectra of that component in adjacent LC fractions, and by observing the behavior of the spectra under changing ionizing conditions. But occasionally no resolution between two or more choices will be possible.

When no choice is possible between one or more functional group options, there are two reporting options; all possibilities can be listed, or the peaks can be reported as unclassified. In either case qualifying remarks need to be made in the "Other" section.

It should be kept in mind that an identification that is limited to an either/or choice, but which is specific to that choice, gives a great deal more information about the unknown than does an "Unclassified" listing. Thus it would in general be preferrable to list both possibilities in the category section, and explain in either the subcategories and specific compounds section, or under Other.

In actual practice it will be found that this degree of decision making is not often needed. Mixtures too complex to allow identification will very often have other justification for listing one, or perhaps all of the possible categories of the not quite identifiable component.

Multiple category listing of single spectral components should be used only as a last resort, and then only if the component is a significant one in the fraction. Every effort should be made to narrow the identification down to a specific type.

E. Additional Categories

The standard Level 1 categories given in Table 14 are to be used for sample description whenever possible, but they should not be used when rigid adherence to them might be misleading. There are many different materials that, when analyzed by direct insertion probe LRMS, may be recognized for what they are; yet they may be difficult to classify into the standard categories, or may be misleading if so classified. The thermal decomposition products of cellulose and of some synthetic polymers are examples of materials of this sort. The analyst may very well encounter other materials that exhibit similar reporting difficulties.

When material of this sort is recognized, it is preferable to report it for what it is recognized to be, rather than to force it into the standard categories. The material should be reported at the category level, with intensity value and if possible, with molecular wieght range also. Supporting information will be needed elsewhere on the reporting form, either in subcategories and specific compounds, or in the Other space.

F. Subcategory and Specific Compounds Reporting

The two levels of subcategory and specific compounds are grouped together in the second section of the LRMS report. This is the region of greatest specificty in the report; the most accurate detail that can be obtained in the analysis of the LC fraction is listed here. This section gives the evidence for the category assignments and at the same time expands the category assignment to greater detail. All materials reported in the category section should be backed up here, or in the "other" section below. The listing should be ordered on abundance, subordered on molecular weight. In the instance of a molecular weight range being reported, the lowest mw of the range should be used for ordering.

No distinction should be shown in the listing between subcategory and specific compound identifications, as there is in fact a continuous gradation from one to the other in the precision with which assignments can be made. No greater precision should be specified than can be substantiated by the LRMS data, but all of the available information should be reported. A molecular weight value or range of values for most categories will also indicate a chemical composition or range of compositions. Both should be listed:

Intensity	Subcategory	m/e	Composition
100	Alkanes	198-268	$C_{14}H_{30}-C_{19}H_{40}$
100	Alkyl Benzenes	106-148	$C_8H_{10}-C_{11}H_{16}$

Specific isomers should be listed only when they can be established as such. The fact that the reference spectrum used for identification was of a specific isomer, does not usually prove that the unknown is that isomer, unless the other possible isomers are also given and are different enough to be distinguished in the mixed species spectra that are being evaluated. Thus, a naphthol should be reported as:

100	Naphthol	144	$C_{10}H_8O$

or as

100	Hydroxy Napthalene	144	$C_{10}H_8O$

and not as

100	1-Naphthol	144	$C_{10}H_8O$

Identifiable isomers should be listed as such:

100	di-n-butyl phthalate	278	$C_{16}H_{22}O_4$

PAH and other polycyclic compounds are rarely distinguishable isomerically, and the names of the isomers of a given composition may be too different to combine in the listing. However, the number of rings in the compound is determined by the composition, which is in turn indicated by the molecular weight. This provides a recognizable form of information that is as specific as can be justified from the spectra. Representative names of compounds may be shown in parenthesis, to indicate that they are representative only:

100	4 ring PAH (e.g., Chrysene, Benzanthrenes)	228	$C_{18}H_{12}$

The interpretation aid tables of Chapter III can be used for these assignments.

There will often be opportunity to group listings without loss of information. This can make the form more readable and save time for the analyst. An example of such grouping is

100	Alkyl 3 ring PAH	192-220	$C_{15}H_{12}$-$C_{17}H_{16}$
10	Alkyl 3 ring PAH	234-262	$C_{18}H_{18}$-$C_{20}H_{22}$

This listing is more efficient than the six separate listings that would be required without grouping, yet the information content is the same.

G. The Other Section of the Report

The "Other" section of the LRMS Level 1 report is the area that is to be used for presentation of information that is not covered in the more specific sections of the report, and/or for any remarks that are relevant to the analysis of the specific fraction under evaluation.

Material listed as Unclassified in the category section should have amplifying information listed in the Other section. This information should include the mass and comparative intensity levels of the major unknown peaks, and the temperature of their appearance if from the probe.

Any other material not accounted for in the subcategory and specific compounds section should be listed here. This should include intensity levels and mass peak ranges for low concentration level materials too complex to be accountable otherwise.

H. Examples

The results obtained for the analysis examples of Chapter IV are shown in the Level 1 LRMS Report format in Figures 26, 27, 28, and 29. Each example is reported as though the illustration spectra were a complete set for that LC fraction.

LRMS REPORT

SAMPLE: Example 1, Chapter IV

Major Categories

Intensity	Category	MW Range
100	Phenols	186-220
100	Ketones	208
100	Ethers	208

Sub-Categories, Specific Compounds

Intensity	Category	m/e	Composition
100	Biphenol	186	$C_{12}H_{10}O_2$
100	Anthraquinone	208	$C_{14}H_8O_2$
100	Methoxy Phenanthrene	208	$C_{15}H_{12}O$
100	Alkyl Phenols	220	$C_{15}H_{24}O$

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Other

FIGURE 26 SAMPLE LEVEL 1 LRMS REPORT, EXAMPLE 1

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TOP

LRMS REPORT

SAMPLE: Example 2, Chapter IV

Major Categories

Intensity	Category	MW Range
100	Fused Alternate, Nonalternate Hydrocarbons	<216

Sub-Categories, Specific Compounds

Intensity	Category	m/e	Composition
100	3 Ring PAH (e.g., phenanthrene)	178	$C_{14}H_{10}$
100	3 Ring PAH (fluorene)	166	$C_{13}H_{10}$
100	3 Ring PAH (acenaphthene)	154	$C_{12}H_{10}$
100	3 Ring PAH (acenaphthylene)	152	$C_{12}H_8$
10	Alkyl 2 Ring PAH (naphthalenes)	142-170	$C_{11}H_{10}$-$C_{13}H_{14}$
10	Alkyl 3 Ring PAH (or biphenyls)	168-182	$C_{14}H_{12}$-$C_{15}H_{14}$
10	Methyl Fluorene	180	$C_{14}H_{12}$
10	Methyl 3 Ring PAH (e.g., Phenanthrenes)	192	$C_{15}H_{12}$

10 Lines to End of Typing Area

Other

FIGURE 27 SAMPLE LEVEL 1 LRMS REPORT, EXAMPLE 2

For Page Numbering

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TOP

LRMS REPORT

SAMPLE: Example 3, Chapter IV

Major Categories

Intensity	Category	MW Range
100	Fused Alternate, Nonalternate Hydrocarbons	<216
100	Fused Alternate, Nonalternate Hydrocarbons	>216
10	Aromatic Hydrocarbons	Unk.
10	Unclassifiable	

Sub-Categories, Specific Compounds

Intensity	Category	m/e	Composition
100	Alkyl Naphthalenes	170-224	$C_{13}H_{14}$-$C_{17}H_{20}$
10	Alkyl Naphthalenes	142-282	$C_{11}H_{10}$-$C_{21}H_{30}$
10	Alkyl Benzenes	>134	$>C_{10}H_{14}$
	(Alkyl naphthalenes includes several		
	degrees of unsaturation in the alkyl		
	component.)		

Other

10 - Each: possible 3 ring PAH, m/z 152, 166; methyl 3 ring PAH, compositions $C_{12}H_8$ to $C_{14}H_{12}$. 10 each: many unclassifiable peaks, m/z 130 to 266. 1 each: other peaks as high as m/z 290.

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FIGURE 28 SAMPLE LEVEL 1 LRMS REPORT, EXAMPLE 3

For Page Numbering

LRMS REPORT

SAMPLE: Example 4, Chapter IV

Major Categories

Intensity	Category	MW Range
100	Fused Alternate, Nonalternate Hydrocarbons	<216
100	Aromatic Hydrocarbons	182-196
100	Esters	Unk.
100	Heterocyclic Nitrogen Compounds	131-201
10	Aromatic Hydrocarbons	168-210
10	Unclassified	150-220

Sub-Categories, Specific Compounds

Intensity	Category	m/e	Composition
100	Alkyl 3 Ring PAH, C_1, C_2	180-194	$C_{14}H_{12}$-$C_{15}H_{14}$
100	3 Ring PAH (e.g., phenanthrene)	178	$C_{14}H_{10}$
100	Phthalate, not Specifically Identifiable		
100	Methyl 3 Ring PAH	192	$C_{15}H_{12}$
100	Alkyl Indoles	145-159	$C_{10}H_{11}N$-$C_{11}H_{13}N$
100	Alkyl Biphenyls	182-196	$C_{14}H_{14}$-$C_{15}H_{16}$
10	Alkyl Indoles	131	C_9H_9N
10	Alkyl Indoles	173-201	$C_{12}H_{15}N$-$C_{14}H_{19}N$
10	Alkyl 3 Ring PAH	166-208	$C_{13}H_{10}$, $C_{16}H_{16}$
10	Alkyl Biphenyls	168-210	$C_{13}H_{12}$, $C_{16}H_{18}$
10	Alkyl Naphthalenes	142,198	$C_{11}H_{12}$, $C_{15}H_{20}$
10	3 Ring PAH (acenaphthylene)	152	$C_{12}H_8$
10	4 Ring PAH (e.g., pyrene)	202	$C_{16}H_{10}$

Other

10 each: many unclassifiable peaks, m/z 160 to 200.

FIGURE 29 SAMPLE LEVEL 1 LRMS REPORT, EXAMPLE 4

REFERENCES

1. Lentzen, D.E., D.E. Wagoner, E.D. Ester, and W.F. Gutknecht, "IERL-RTP Procedures Manual: Level 1 Environmental Assessment (2nd Ed.)," EPA 600/7-78-201, NTIS PB 293-795, (1978).

2. Freudenthal, J., "Environmental Application of Mass Spectrometry," Advances in Mass Spectrometry, 7, 1659 (1978).

3. Alford, A., Biomed. Mass Spectrom., 4, 1 (1977).

4. Hertz, H.S., W.E. May, S.A. Wise, S.N. Chesler, Anal. Chem., 50, 428A (1978).

5. Stauffer, J.L., Unpublished Data.

6. McLafferty, F.W., Interpretation of Mass Spectra, 2nd Ed., W.A. Benjamin, 1973.

7. Brown, R.A., Anal. Chem., 23, 430 (1951).

8. Gallegos, E.J., J.W. Green, L.P. Lindeman, R.L. LeTourneau, R.M. Teeter, Anal. Chem., 39, 1833 (1967).

9. Lumpkin, H.E., B.W. Thomas, A. Elliot, Anal. Chem., 24, 1389 (1952).

10. Clerc, R.J., A. Hood, M.J. O'Neal, Jr., Anal. Chem., 27, 868 (1955).

11. Lumpkin, H.E., Anal. Chem., 28, 1946 (1956).

12. Field, F.H., S.H. Hastings, Anal. Chem., 28, 1248 (1956).

13. Lumpkin, H.E., Anal. Chem., 30, 321 (1958).

14. Lumpkin, H.E., B.H. Johnson, Anal. Chem., 26, 1719 (1954).

15. Hastings, S.H., B.H. Johnson, H.E. Lumpkin, Anal. Chem., 28, 1243 (1956).

16. Stevens, C.M., Rev. Sci. Instrum., 24, 148 (1953).

17. Monteiro, L. Fraser, R.I. Reed, J. Mass Spec. and Ion Physics, 2, 265 (1969).

18. Knorr, Fritz J., J.H. Futrell, Anal. Chem., 51, 1236 (1979).

19. Grigsby, R.D., S.E. Scheppele, G.J. Greenwood, P.L. Spraggins, T.D. Marriott, Proceedings, 25th Annual Conference on Mass Spectrometry and Allied Topics, Washington, D.C., May 29 - June 3, 1977, p. 154.

REFERENCES (Continued)

20. *Eight Peak Index of Mass Spectra*. 4 volumes, 2nd ed. Publisheed by Mass Spectrometry Data Centre, AWRE, Aldermaston, Reading, RG7 4PR, U.K., 1974.

21. Heller, S.R., and G.W.A. Milne. *EPA/NIH Mass Spectral Data Base*. 5 volumes, U.S. Department of Commerce/National Bureau of Standards NSRDS-NBS 63, December 1978.

22. Hites, R.A., V. Lopez-Avila, *Anal. Chem.*, 51, 1452A (1979).

23. Games, L.M., R.A. Hites, *Anal. Chem.*, 49, 1433 (1977).

24. Grigsby, R.D., R.H. Cole, W.G. Fox, G.M. Gable, Proceedings, 18th Annual Conference on Mass Spectrometry and Allied Topics, San Francisco, June 1979, p. B273.

25. Grigsby, R.D., C.O. Hansen, D.G. Mannering, W.G. Fox, R.H. Cole, *Anal. Chem.*, *43*, 1135 (1971).

26. Weinkam, R.J., Huey-shin Lin, *Anal. Chem.*, *51*, 972 (1979).

27. Crawford, L.R., J.D. Morrison, *Anal. Chem.*, *40*, 1464 (1968).

28. Atwater (Fell), Barbara L., R. Venkataraghavan, F.W. McLafferty, *Anal. Chem.*, 51, 1945 (1979).

29. McLafferty, F.W., Mass Spectral Correlations, ACS Advances in Chemistry Series *40*, Washington, 1963.

30. Erikson, M.D., D.L. Newton, E.D. Pellizzari, K.B. Tomer, *J. of Chrom. Sci.*, *17*, 449 (1979).

31. Quayle, A., R.E. Reed, Mass Spectrometry, *Interpretation of Organic* Spectra, D.W. Mathieson, ed. Academic Press, New York, 1965.

32. Stauffer, J.L., P.L. Levins, J.E. Oberholtzer, in *Carcinogenesis, A Comprehensive Survey, Vol. 3*, Freudenthal, R.I., and P. Jones, (eds), Raven Press, New York, New York, 1976.

33. Crawford, L.R., J.D. Morrison, *Anal. Chem.*, 40, 1469 (1968).

34. Giger, W., M. Blumer, *Anal. Chem.*, *46*, 1663 (1974).

35. Blumer, M., *Scientific American*, *234*, March 1976.

36. Fox, M.A., S. Olive, *Science*, *205*, 582 (1979).

37. Snook, M.E., in *Carcinogenesis, A Comprehensive Survey, Vol. 3*, Jones, P.W., R.E. Freudenthal, eds. Raven Press, New York, 1978.

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TOP

REFERENCES (Continued)

38. Estes, E.D., F. Smith, and D.E. Wagoner, "Interagency Energy/Environmental R & D Program Report," Level 1 Environmental Assessment Performance Evaluation, EPA-600/7-79-032, February 1979.

39. Cleland, J.G. and G.L. Kingsbury, "Multimedia Environmental Goals for Environmental Assessment," Volume II, EPA-600/7-77-136b, November 1977.